



Environmental Physiology of
Cultured Early-Stage
Southern Rock Lobster
(*Jasus edwardsii* Hutton, 1875)
Larvae

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Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information in duly acknowledged in the thesis, and to the best of the candidate's knowledge and belief, no material previously published or written by another person except where due acknowledgment is made in the text of the thesis.



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Abstract

The aim of this project was to define more clearly the culture conditions for the propagation of the southern rock lobster (*Jasus edwardsii*) in relation to environmental bioenergetic constraints.

The effects of temperature and photoperiod on the first three stages of development were first studied in small-scale culture experiments. Larvae reared at 18°C developed faster and reached a larger size at stage IV than larvae cultured at 14°C. Development through stage II was shorter under continuous light. However, the pattern of response to photoperiod shifted at stage III when growth was highest in all the light/dark phase treatments than under continuous light.

The influence of temperature and light intensity in early-stage larvae was further investigated through behavioural and physiological studies. Results obtained in stages I, II and III larvae indicated an energetic imbalance at high temperature (~22°C). The behavioural response of stage I larvae to light intensity suggested that light may be used to control behaviour in culture conditions. Early-stage larvae showed higher oxygen consumption, nitrogen excretion, and feed intake under light than in the dark. This may be due to the demonstrated increased activity under light conditions.

A technique based on the chemical immobilisation of larvae was developed to assess the effect of temperature on the standard metabolic rate and the energetic cost of swimming in phyllosomas. Estimates of larval locomotor activity at different temperatures obtained through measurements of oxygen consumption were in agreement with behavioural response under the same conditions.

The water quality requirements of *J. edwardsii* larvae were determined for dissolved oxygen, salinity, and ammonia. A critical oxygen tension of 4.3 ml O₂ l⁻¹ was found for stage I larvae at 18°C. Stage I larvae were found to be stenohaline and a 3 ppt departure from normal salinity (34 ppt) during culture had a significant effect on growth. The cost of osmoregulation was examined in newly-hatched larvae and in stage I phyllosomas acclimated or not to sub-normal

salinities. Safe levels of total ammonia concentration determined for stages I, II, III, and IV were 2.65 mg l⁻¹, 3.83 mg l⁻¹, 4.37 mg l⁻¹, and 2.98 mg l⁻¹, respectively.

The results documented throughout this thesis highlighted the significance of environmental manipulation to achieve greater survival and growth during the larval development of *J. edwardsii*. In addition, information on the environmental physiology and behaviour of early-stage larvae provided an insight into an integrated approach, which at term will allow for the definition of system and dietary requirements of all developmental stages.

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Chapter 1

General Introduction

1.1 Background

Following in the footsteps of Japan and New Zealand, Australia is showing a growing interest in spiny lobster aquaculture. As with many aquaculture species, the high market value of spiny lobster and its declining wild fisheries resources around the world make it a prime candidate for mariculture (Kittaka, 1994). In the last decade, researchers have developed techniques for the mass rearing of wild caught juveniles or pueruli of several species. Culture trials carried out in tanks, pens, and cages (Chittleborough, 1974; Rahman *et al.*, 1994; Assad *et al.*, 1996; Hooker *et al.*, 1997; Lozano-Alvarez, 1996) have lead to promising results in terms of profitability (Rahman and Srikrishnadhas, 1994) providing that a cost-effective diet can be developed (Jeffs and Hooker, 2000). In Australia, extensive research is currently under way on the nutritional requirements of spiny lobsters (Crear *et al.*, 2000; Glencross *et al.*, 2001). However, there are obvious constraints with the harvest of wild pueruli for aquaculture purposes. Naturally, the practice is controversial with the fishing industry and ways of regulating exploitation must be implemented. The commercial harvest of pueruli is currently taking place in New Zealand, in the Australian state of Tasmania, and is in development in the Republic of South Africa. Although not documented, there are reports of puerulus collection for the growing of spiny lobsters in India, Korea, Japan, Taiwan, Vietnam, the Philippines, and Chile (David Mills, personal communication). Of those countries, only New Zealand and Tasmania have implemented regulatory measures for the management of puerulus collection. In New Zealand, these regulations consist in the exchange of a one tonne fisheries quota for 40,000 pueruli, while in Australia, permits for the harvest of 50,000 pueruli require that after one year of capture the holders of such permits release 5% of the initial catch and 20% of the remaining numbers. Other than legal constraints, a spiny lobster aquaculture industry is likely to be limited by the inter-annual fluctuations in recruitment previously reported in *Jasus edwardsii* (Gardner *et al.*, 1998) and in other panilurid species such as *Panulirus cygnus* in Western Australia (Phillips

and Pearce, 1989). Extended periods of low recruitment have been observed in Hawaii and have resulted in the failure of the *Panulirus marginatus* fishery in the north-western region of the archipelago (Haight, 1997). Therefore, the inconsistency in seed availability may result in the inability of the producers to predict output and therefore to secure markets, and funding or loans from financial institutions. In Australia, these legal and natural constraints intrinsic to spiny lobster fisheries have prompted the development of research programs on the propagation of several panilurid species, which if successful, would ensure the sustainability and durability of both aquaculture and wild fisheries sectors.

Japan has been leading research into spiny lobster propagation for over 50 years and this work was reviewed by Kittaka (1994). From this extensive experience, Japanese researchers have consistently succeeded in producing small numbers of post-larvae (up to 325 pueruli) of various panilurid species of the *Jasus* (Kittaka, 1988; Kittaka *et al.*, 1988; Kittaka *et al.*, 1997), *Panulirus* (Kittaka and Kimura, 1989; Yamakawa *et al.*, 1989; Matsuda and Yamakawa, 2000; Sekine *et al.*, 2000), and *Palinurus* (Kittaka and Ikegami, 1988) genera. However, the propagation of spiny lobsters on a commercial scale is still not technically feasible.

J. edwardsii (southern rock lobster) is a temperate species distributed throughout southern Australia and in New Zealand (Edgar, 1997). After hatching in coastal waters (MacDiarmid, 1985), the planktonic phyllosoma of the southern rock lobster can reach distances of more than 900 km offshore (Booth, 1994). The pelagic larval phase is estimated to last between 13 and 24 months (Bruce *et al.*, 2000) during which, growth to the puerulus nektonic stage takes place through 11 distinct developmental stages (Lesser, 1978). Research on the propagation of *J. edwardsii* was initiated in the 1990's in New Zealand (Booth, 1996) and has contributed to the development of novel culture techniques for spiny lobster larvae (Illingworth *et al.*, 1997). Despite these recent advances, researchers both in New Zealand and Japan have encountered tremendous difficulties in rearing a handful of *J. edwardsii* larvae through up to 17 instars and for as long as 416 days until they moulted into the puerulus post-larval stage (Kittaka, 1994; Booth, 1996). Additionally, the survival of pueruli through to the first juvenile moult

remains low (Kittaka, 1994). In *Panulirus japonicus*, the duration and body size of the puerulus stage was reported to be influenced by the nutritional and environmental history experienced throughout the larval phase (Sekine *et al.*, 2000). A study by Jeffs *et al.* (2000) also confirmed the importance of lipid reserves for post-settlement development in *J. edwardsii* pueruli. Therefore, considering the environmental consistency experienced by *J. edwardsii* during larval development (i.e. 'phyllosoma water', Bruce *et al.*, 2000), the performance of potential new-recruits is likely to be dictated by feed availability, as is the case in fish larvae (Canino *et al.*, 1991). In addition to this, all abiotic factors that can be controlled (e.g. salinity, dissolved oxygen, ammonia) and manipulated (e.g. temperature, photoperiod, light intensity) in a hatchery situation can influence the energetic balance and consequently the survival and growth of decapod larvae (Pandian and Vernberg, 1997). Therefore, the successful propagation of *J. edwardsii* along with other panilurid species will require a thorough understanding of the environmental biology and ecology of phyllosoma.

1.2 Objectives and scope of the study of environmental physiology in phyllosoma

At the commencement of this project in 1998, little information was available on the environmental requirements for propagation of *J. edwardsii* larvae. Therefore, the research presented in this thesis aimed to further define culture conditions in order to enhance survival and growth rate. Since then, studies by Moss *et al.* (1999) and Tong *et al.* (2000) have demonstrated the effects of light intensity and temperature, respectively, on the development of early- and mid-stage *J. edwardsii* larvae. The influence of temperature and light intensity on early-stage larvae are also documented in the present thesis together with the effects of photoperiod and water quality parameters such as dissolved oxygen, salinity and total ammonia, which have, so far, received little attention in spiny lobster phyllosomas. The response of phyllosoma larvae to these environmental variables was described in terms of survival, growth, physiology, and behaviour. Given the difficulty in rearing spiny lobster larvae (see survival rates in Kittaka, 2000) the present thesis focused essentially on early developmental stages. The methods documented here were developed with the intention to be easily transferable to more advanced stages once survival through the early-stages is improved.

1.2.1 Survival and growth

The survival and growth of early-stage phyllosomas was examined using small-scale experimental culture systems to rear larvae at different temperatures, photoperiods, salinities, and total ammonia concentrations. Each of these factors have been reported to significantly influence development in decapod larvae (Armstrong, 1978; Matsuda and Yamakawa, 1997; Gardner and Maguire, 1998; Kumlu *et al.*, 2000; among others). Therefore, the objective of this work was to define optimal culture conditions for larval rearing and determine possible ontogenic changes in response to environmental factors during the first stages of development.

1.2.2 Physiology

So far, only a few studies have dealt with indicators such as oxygen consumption in palinurid larvae (Belman and Childress, 1973) despite their comparative value in understanding environmental physiology (Willmer *et al.*, 2000). Oxygen consumption, nitrogen excretion, and feed intake were measured in stages I, II, III and V larvae (Appendix I) with techniques adapted from the vast literature available on the topic (see reviews by Omori and Ikeda, 1984, Båmstedt *et al.*, 2000, and Ikeda *et al.*, 2000). The objective of this approach was to define culture conditions for early-stage *J. edwardsii* larvae through the assessment of their physiological response to different temperatures, light intensities, salinities, or under progressive hypoxia. This approach also provided scope for the detection of possible ontogenic changes in response to temperature and light intensity during early development (Lemos and Phan, 2001). In addition, information on the physiological response of *J. edwardsii* larvae to environmental variables were expected to provide supportive bioenergetics evidence for the results that were obtained when growing larvae in small-scale experimental culture systems.

1.2.3 Behaviour

Factors such as temperature, light intensity, and salinity are all determinant cues for the orientation and the locomotor activity of decapod larvae (Sulkin, 1984). Field surveys (Booth, 1994; Bruce *et al.*, 2000) have provided to date the only information available on the behavioural response of mid- and late-stage *J. edwardsii* phyllosoma to environmental factors. Considering the significance of locomotor activity in the measurements of oxygen consumption in zooplankton (Halcrow and Boyd, 1967; Torres and Childress, 1983; Buskey, 1998), it was essential to describe the behaviour of phyllosoma under different environmental conditions for the interpretation of their metabolic response. A technique based on the chemical immobilisation of phyllosoma was also assessed to determine the standard metabolism and the energetic cost of locomotory activity in newly-hatched larvae. In addition, an understanding of the environmental cues driving

depth regulation in phyllosoma is necessary to control their behaviour in a culture situation. The environmental control of behaviour may prevent responses such as aggregation or swimming towards tank walls, which may enhance mortality by promoting disease transmission, cannibalism, and reducing the encounter rate with prey.

1.3 Structure of the thesis

This document contains a general introduction, six research chapters, and a general discussion. At the time of submission for review, none of the results presented throughout this document have been published in scientific journals. However, each chapter is organised as a journal manuscript and it is the intention of the author to disseminate this information through publication following review of the thesis. The outcome of the experiments outlined in Chapter 2 and 4 were presented at conferences:

Bermudes, M., 1999. Effect of photoperiod on survival, growth, feeding and cannibalism in early developmental stages of *Jasus edwardsii* phyllosoma larvae. In: Proceedings of the 1999 FRDC rock lobster propagation workshop, Jan 29th – 31st, Hobart. Tasmanian Aquaculture and Fisheries Institute, University of Tasmania, p. 19.

Bermudes, M. and Powell, M., 2000. Estimation of the swimming response of rock lobster (*Jasus edwardsii*, Decapoda: Crustacea) larvae to temperature using respirometry. In: Proceedings of the Annual Conference of the Australian Marine Science Association, Sydney, July 11-14, 2000.

The study in Chapter 3 was part of a larger project funded by the Australian Fisheries Research and Development Corporation, and implemented for the development of rock lobster propagation techniques (Crear and Hart, 2001).

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Chapter 2

The effect of temperature
and photoperiod on
survival, growth and feeding
in early-stage larvae

2.1 Abstract

The effect of temperature and photoperiod, and the ontogenic changes in response to these factors was examined in two separate experiments from observations of survival, intermoult period, moult increment and feeding activity at stage I, II and III. Larvae were reared in static water systems in groups of 20-21 animals. Phyllosomas were cultured at 10.5, 14.3, 18.2 and 21.5°C in one trial, and under 0, 6, 12, 18 and 24 hours of light/day (L) in the other. Rearing was terminated at stage II in larvae tested at 10.5°C and 21.5°C. In stage I larvae the Bělehrádek's expression was fitted to the relationship between intermoult period and temperature ($V = 48.716 (T - 9.425)^{-0.579}$). Survival was not affected by temperature at stage I, although at 21.5°C, all larvae except one died at stage II. Post-moult growth at stage I was greatest at 18.2°C. The rate of development and moult increment remained greater at 18.2°C than at 14.3°C through to stage III. Consumption of *Artemia* nauplii by stage I larvae was highest but not different at 18.5°C and 21.5°C. Photoperiod did not affect survival through the first three stages but had a marked effect on intermoult period, growth and feeding. The response to increasing photoperiod changed during development. Stage I and II larvae tended to develop at a greater rate and feed more under increasing light phase whereas stage III larvae required a light/dark phase to achieve higher rate of development and growth. Results were compared with conclusions previously reached in New Zealand on the effects of temperature and light on *J. edwardsii* larvae. Discrepancies of outcome between these studies and the present work are discussed with respect to differences in experimental approach.

2.2 Introduction

Both temperature and photoperiod are parameters easily manipulated in the laboratory and they have major effects on the survival and growth of crustacean larvae in culture conditions. In poikilotherm organisms such as decapod larvae, temperature determines basal metabolic rate and may influence the production of new tissue, moulting, feeding activity and locomotor activity (Clarke, 1987). As such, temperature was reported to affect survival (Kurata, 1960; Kumlu *et al.*, 2000), rate of development (Templeman, 1936; Paul and Paul, 1999) and growth (Rothlisberg, 1979; Johns, 1981; Minagawa, 1990) of larval crustaceans. As opposed to temperature, photoperiod does not have a direct effect on the metabolism of animals. However, parameters of light such as intensity (Forward *et al.*, 1984), spectral composition (Ritz, 1972; Gardner, 1996), and angular distribution (Schalleck, 1942) can all affect locomotor activity in decapod larvae, while light intensity can influence feeding in *Jasus edwardsii* phyllosoma (Moss *et al.*, 1999). Therefore under culture conditions, variations in the duration of the light and the dark phase could considerably affect the energetic balance of light responsive larvae such as phyllosomas (Ritz, 1972; Mikami, 1995). Shifts in photoperiod have in fact been reported to influence survival (Minagawa, 1994), rate of development (Dawirs, 1982), and growth (Templeman, 1936) in decapod larvae.

The present study aimed to examine the influence of temperature and photoperiod on the survival, rate of development, growth and feeding activity of the first three larval stages of *J. edwardsii*. The continuous rearing of phyllosoma from a few hours after hatching through to the moult into stage IV was conducted in static water systems allowing for the exposure of animals to different temperatures or photoperiods. The results obtained with this method are discussed with reference to work previously published on aspects of the response to temperature (Tong *et al.*, 2000b) and light intensity (Moss *et al.*, 1999) in this species.

2.3 Material and methods

2.3.1 *Larvae*

Ovigerous females were brought from the east coast of Tasmania to the TAFI Marine Research Laboratories in Hobart (Australia), in June 1998. Females were kept in indoor tanks on a flow-through system and fed regularly with squid and mussels until larval release. The larvae hatched in November at an ambient temperature of 14.5°C. For temperature and photoperiod trials, larvae were collected on the same day from two and three broods, respectively, with initial mean (\pm SE) larval length of 2.08 ± 0.03 mm and 2.15 ± 0.03 mm, respectively. Larvae were reared through the first 3 stages of development described by Lesser (1978) (see also Appendix I) and there was one instar per stage.

2.3.2 *Culture conditions*

Larvae were reared in shallow plastic bowls with 200 ml of sea water and 25 ppm of oxytetracycline (Engemycin 100, Intervet, Australia) (Gardner and Northam, 1997; Appendix II). Antibiotics were used to avoid proliferation of opportunistic bacteria that could interfere with the effect of temperature and photoperiod. In events of high mortality, samples of moribund larvae were prepared onto TCBS medium and sent to the Animal Health Laboratory of the Tasmanian Department of Primary Industries, Water and Environment (Kings Meadows, Australia) for microbiological analysis. There were five bowls per treatment and each bowl was initially stocked with 20 (10 from each of two broods) and 21 (7 from each of three broods) newly-hatched larvae in the temperature and photoperiod trials, respectively. Larvae were transferred to clean bowls and water with fresh oxytetracycline every second day and they were fed daily to satiation on newly-hatched *Artemia* nauplii enriched for 24 hours with DC Super Selco (INVE, Belgium), and *T. Isochrysis galbana* on alternate days.

2.3.2.1 Temperature trial

Phyllosomas were reared under four temperatures (mean \pm SD, daily observations): 10.5 ± 0.5 , 14.3 ± 0.3 , 18.2 ± 0.5 and $21.5 \pm 0.3^\circ\text{C}$, which were achieved in water baths equipped with thermostats. Larvae were cultured under 24 h of light per day with 20 watt quartz halogen lights reflected on a white ceiling above the culture vessels (200 lux). Rearing was terminated at stage II in the 10.5°C and 21.5°C treatments due to slow growth and high mortality, respectively.

2.3.2.2 Photoperiod trial

Larvae were reared under five photoperiods: 0, 6, 12, 18 and 24 h light per day (0L, 6L, 12L, 18L and 24L). The different light phases were achieved in dark chambers with 20 watt quartz halogen lights on timers. Lights were reflected on a white ceiling above the culture vessels (200 lux). This diffuse lighting resulted in the even spatial distribution of larvae and Artemia thus avoiding situations where photoresponsive larvae and/or preys are drawn away from each other (Mikami and Greenwood, 1997). Despite the experiment being conducted in an air-conditioned room, slight fluctuations in temperature from 17.5°C to 18.5°C were expected between treatments. Therefore, hourly records of temperature were taken in each chamber with data loggers. Mean \pm SD (computed from mean daily records) temperatures were 18.28 ± 1.16 , 18.37 ± 1.23 , 18.06 ± 1.32 , 18.59 ± 1.17 and $18.53 \pm 1.05^\circ\text{C}$ in 0L, 6L, 12L, 18L and 24L treatments, respectively. There was no statistical difference in temperature between treatments (ANOVA, $F_{4,256} = 1.69$, $P = 0.152$).

2.3.3 Assessment of larval performance

Mortality was recorded daily and expressed as survival to stage II, III and IV as the percentage of larvae stocked at the start of the experiment. The rate of development was measured in terms of stage duration (intermoult period). Intermoult duration of stages I, II and III was monitored at moulting when all exuviae were removed and counted daily. In the photoperiod trial, intermoult

duration was first calculated in degree-day according to the temperatures recorded in each treatment with data loggers. Degree-day data were then expressed in days at 18°C for interpretation and presentation. To examine treatment effects on moult increment, 12 stage I larvae from each brood were measured for initial length and five randomly selected larvae from each replicate were subsequently measured at stage II, III and IV. Total body length was measured from the anterior of the cephalic shield to the end of the telson to the nearest 25 µm (Nikon Profile Projector Model 6C). Larvae were returned to their respective rearing vessel after measurement. Feed intake was determined at mid-stage I in the temperature trial, and at mid-stage I, II and III in the photoperiod trial. Mid-stage I at each temperature was estimated from preliminary experiments and feed intake was assessed on day 5 at 21.5 and 18.2°C, and on day 9 and 20 at 14.3 and 10.5°C, respectively. Daily feed intake was assessed on a same mid-stage day for all photoperiod treatments and at each stage. Stage I, II and III larvae were fed an initial number of around 1000, 1500 and 1500 *Artemia* nauplii per bowl, respectively. The same amount of feed was introduced in three control bowls in each treatment. Estimates of daily feed intake were obtained from the difference in prey number between control and experimental vessels after a 24-h period. Feeding activity of stage I larvae was expressed as daily feed intake of *Artemia* nauplii per larva per day ($\text{Artemia larva}^{-1} \text{ day}^{-1}$).

Cannibalism was observed in the photoperiod trial during stages II and III and records of cannibalised larvae were made in each treatment according to the flow chart presented in Fig. 2.1.

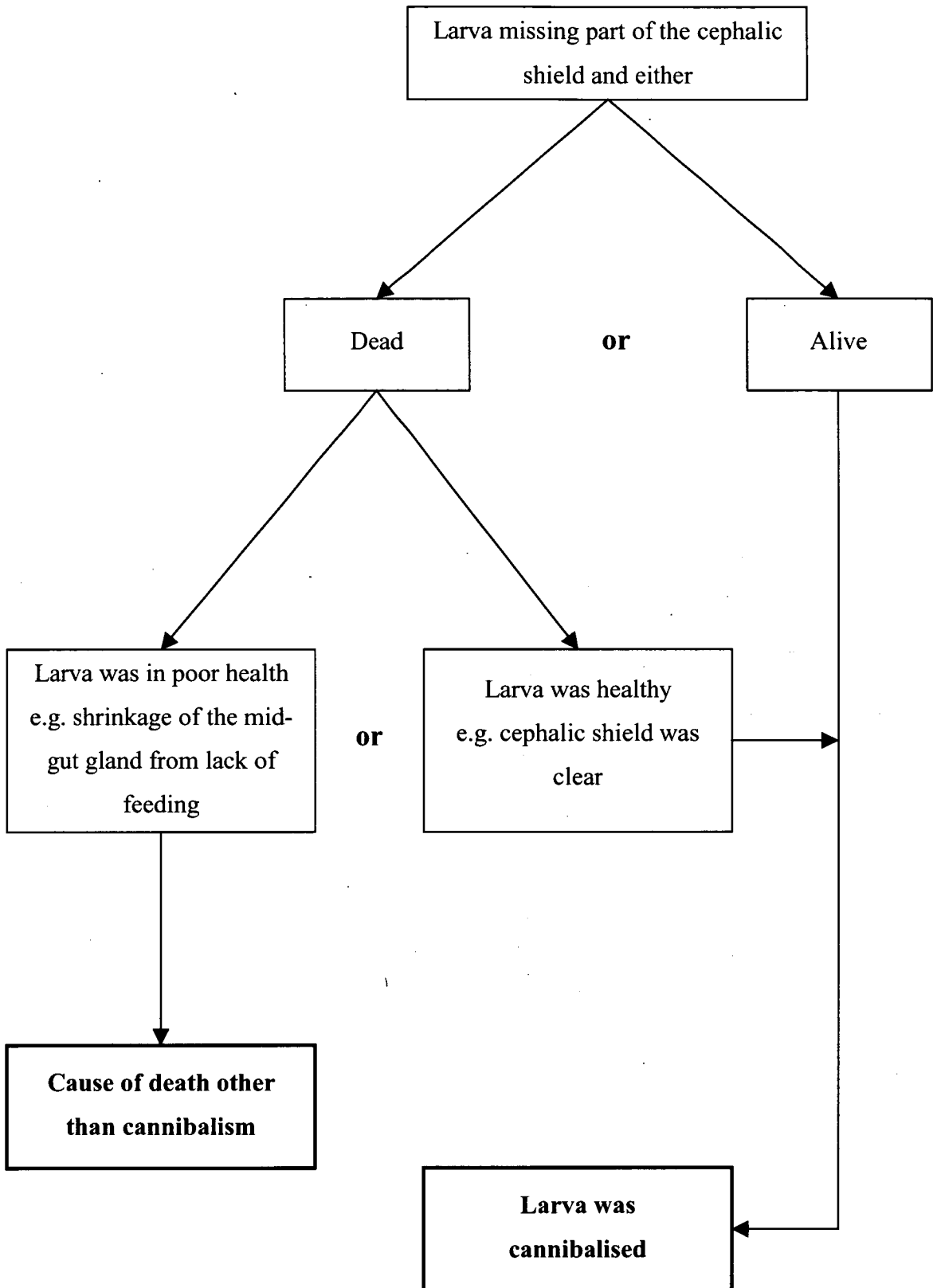


Figure 2.1 Flow chart used to identify larvae that died from cannibalism.

2.3.4 *Statistical analysis*

2.3.4.1 **Temperature trial**

The relationship between intermoult duration of stage I larvae and temperature was fitted with the Bělehrádek's expression (McLaren, 1963):

$$V = a (T + \alpha)^b$$

where V is the duration of the intermoult period (days), T is the temperature ($^{\circ}\text{C}$), and a , b and α are constants. Bělehrádek referred to α as the "biological zero" under which the duration of the intermoult is infinite (McLaren, 1963). Survival, intermoult period and moult increment data were analysed in two steps. The effect of temperature on each of these parameters was first examined at stage I from 10.5 to 21.5 $^{\circ}\text{C}$ by means of Analysis of Variance (ANOVA) followed by Least Significant Difference post-hoc test (LSD). Possible changes in response with development were next examined with repeated measures ANOVA (JMP, 1995) in larvae reared from hatch to the moult into stage IV at 14.3 and 18.2 $^{\circ}\text{C}$. The between subject (temperature treatments) effect was determined with the Pillai's Trace multivariate test (PT). Depending on the significance of the sphericity test, either a univariate unadjusted test (standard F statistic procedure; F stat.) or the Geisser-Greenhouse univariate test (epsilon adjusted for degrees-of-freedom; G-G univ.) were used to examine the within treatment effect of ontogenic development. Survival data were arcsine square root transformed to meet the ANOVA assumption of normality. Daily feed intake data did not meet the ANOVA assumptions of homoscedasticity (Leven Median test) and normality (Kolmogorov-Smirnov test). Therefore, the effect of temperature on feeding activity was analysed with the Kruskal-Wallis one way analysis of variance on ranks (K-W) and the Newman-Keuls pairwise comparison procedure (N-K) was used to detect specific differences between treatments.

2.3.4.2 **Photoperiod trial**

Changes in survival, intermoult period, moult increment and feed intake from stage I to stage III were analysed with repeated measures ANOVA following the procedure described above. Survival data were arcsine square root

transformed to meet the ANOVA assumption of normality. When a significant effect of photoperiod was detected during development the Least Significant Difference post-hoc test (LSD) was used to compare between treatments within a developmental stage. The effect of photoperiod on cannibalism was examined by ANOVA on the total mortality caused by cannibalism at stage II and III.

2.4 Results

2.4.1 Temperature trial

2.4.1.1 Survival

Survival to stage II was not affected by temperature under the four conditions investigated (ANOVA, $F_{3,15} = 2.59$, $P = 0.092$; Table 2.1). There was no overall effect of temperature on the survival of larvae reared beyond stage II at 14.3 and 18.2°C (PT, $F_{1,8} = 0.02$, $P = 0.889$), and in fact, the significant decline in survival observed during development (F stat., $F_{2,16} = 149.36$, $P < 0.001$) was consistent in phyllosoma reared at 14.3 and 18.2°C (F stat., $F_{2,16} = 0.29$, $P = 0.754$). At 21.5°C most larvae died during stage II (Table 2.1). This was possibly caused by starvation as stage II animals reared at 21.5°C were sluggish and did not appear to resume feeding after moulting. In addition, a microbiology report on stage I and II moribund larvae concluded on a normal bacterial flora in larvae cultured at both 18.2 and 21.5°C (Appendix III).

2.4.1.2 Rate of development and growth

At stage I (between 10.5 and 21.5°C)

Stage I larvae took 47.7 ± 1.1 , 20.6 ± 0.2 , 13.4 ± 0.1 and 13.5 ± 0.4 days \pm SD to moult into stage II at 10.5, 14.3, 18.2 and 21.5°C, respectively. The Bělehrádek function described ($r^2 = 0.99$, $F_{1,17} = 2060.36$, $P < 0.0001$) the curvilinear relationship between the duration of larval development at stage I (V in days) and temperature (T in °C) (Fig. 2.2):

$$V = 48.716 (T - 9.425)^{-0.579}$$

In addition, the equation indicated a theoretical biological zero of 9.4°C for larval development of *J. edwardsii* larvae at stage I.

Rearing temperature had a significant effect on body length growth at stage I (ANOVA, $F_{3,15} = 19.35$, $P < 0.0001$). Consequently, stage II larvae were largest at 18.2°C and smallest at 10.5 and 21.5°C (LSD, $P < 0.05$; Fig. 2.3).

From stage I to stage III (at 14.3 and 18.2°C)

The effect of temperature on the duration of the intermoult period at stage I, II and III (PT, $F_{1,8} = 920.68$, $P < 0.0001$; Fig. 2.4) indicated a faster rate of larval development at 18.2°C than at 14.3°C. The intermoult period extended significantly during development (F stat. on stage effect, $F_{2,16} = 77.81$, $P < 0.0001$; Fig. 2.4), and this occurred in larvae reared at 14.3 and 18.2°C (F stat. on temperature x stage effect, $F_{2,16} = 1.28$, $P = 0.304$; Fig. 2.4).

Larvae reared at 18.2°C went through significantly larger moult increments than larvae reared at 14.3°C (PT, $F_{1,8} = 70.32$, $P < 0.0001$; Fig. 2.5). Moult increments decreased with development (F stat., $F_{2,16} = 11.31$, $P < 0.001$), and this trend was similar at both 14.3 and 18.2°C (F stat., $F_{2,16} = 2.14$, $P = 0.150$; Fig. 2.5).

2.4.1.3 Feed consumption at stage I

Daily feed consumption significantly increased with temperature (K-W, $H = 15.3$, $df = 3$, $P < 0.01$; Fig. 2.6). However, there was no significant difference in daily feed intake between larvae reared at 18.2°C and larvae reared at 21.5°C (N-K, $P > 0.05$; Fig. 2.6).

Table 2.2 Survival (percent \pm SD) of *Jasus edwardsii* larvae cultured from hatch through to stage IV at four temperatures. Rearing was terminated at stage II at 10.5°C and no larvae survived past stage III at 21.5°C.

Temperature	Stage of development		
	II	III	IV
10.5°C	83.67 \pm 5.16	—	—
14.3°C	82.67 \pm 16.59	80.71 \pm 16.72	73.94 \pm 14.11
18.2°C	88.19 \pm 7.65	83.29 \pm 10.97	76.33 \pm 12.16
21.5°C	65.86 \pm 16.16	1.00 \pm 2.24*	—
P	> 0.05	> 0.05	> 0.05

* excluded from analysis.

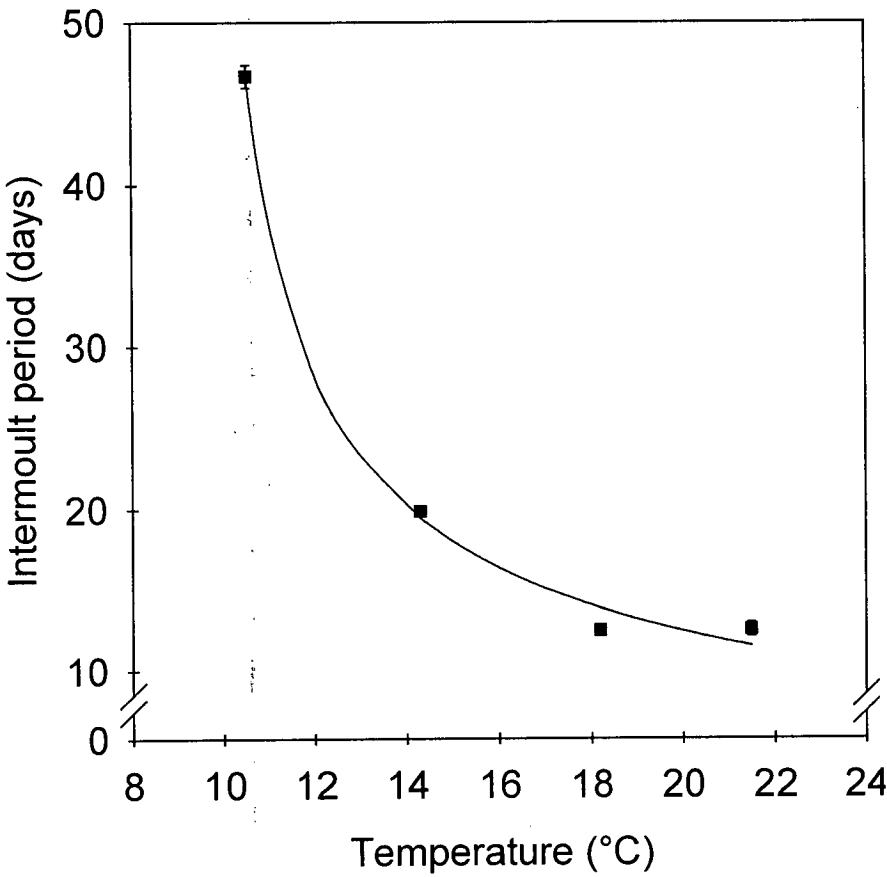


Figure 2.2 Mean (\pm SE) intermoult period of stage I *Jasus edwardsii* larvae reared at 4 temperatures. The Bělehrádek expression was fitted to the raw time data.

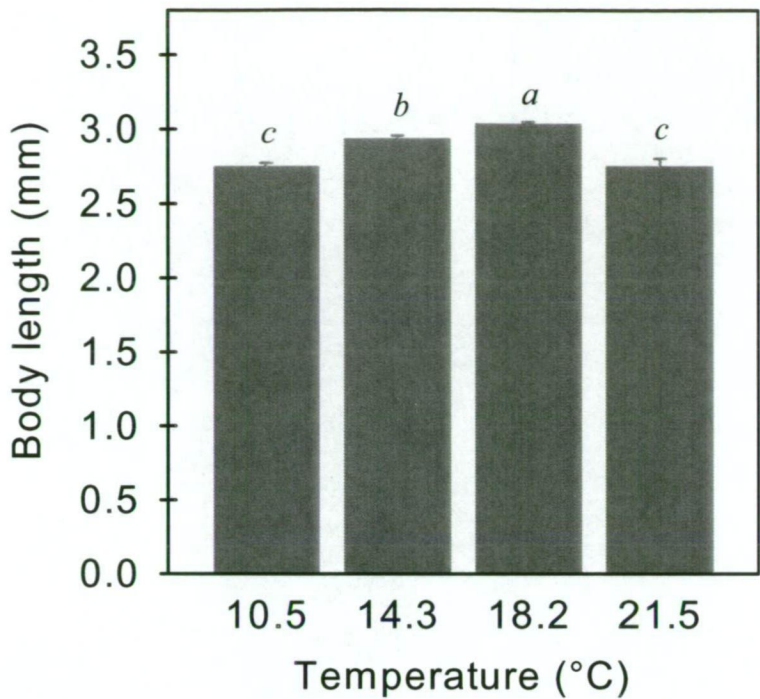


Figure 2.3 Post-moult mean (\pm SE) length of stage II *Jasus edwardsii* larvae reared through stage I at different temperatures. Treatments sharing common letters were not significantly different (LSD, $P>0.05$).

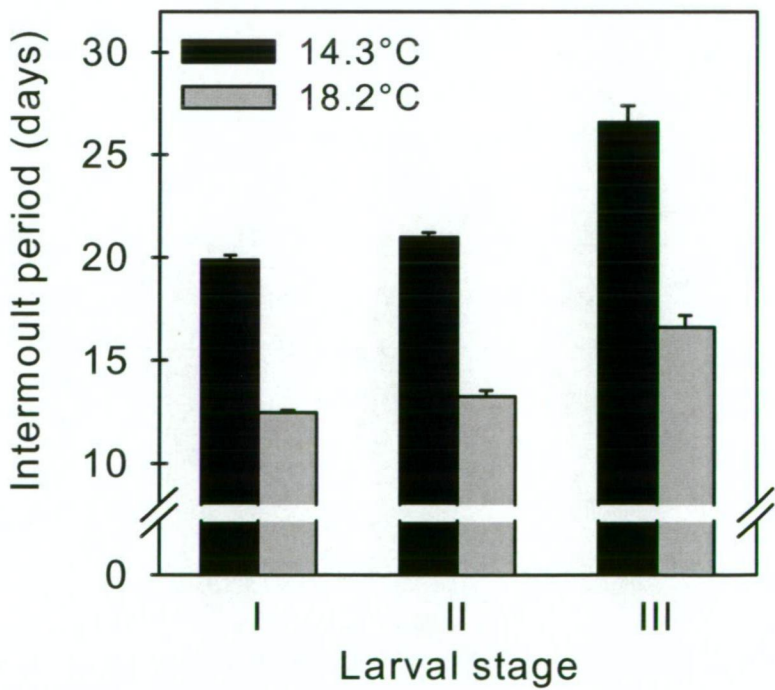


Figure 2.4 Mean (\pm SE) intermoult period of *Jasus edwardsii* larvae reared at 14.3 and 18.2 °C from hatch to moult into stage IV.

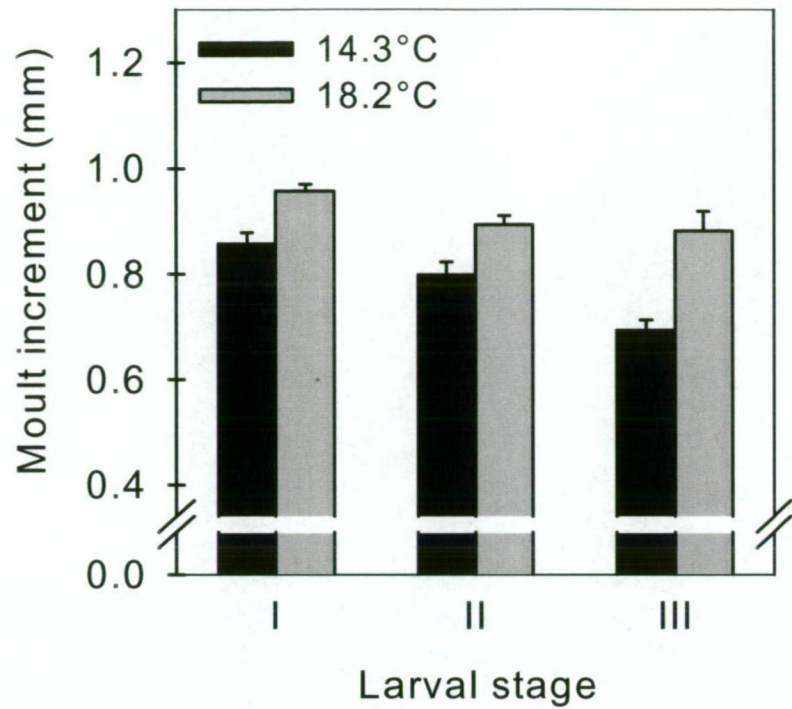


Figure 2.5 Mean (\pm SE) body length moult increment of *Jasus edwardsii* larvae reared at 14.3 and 18.2°C from hatch to moult into stage IV.

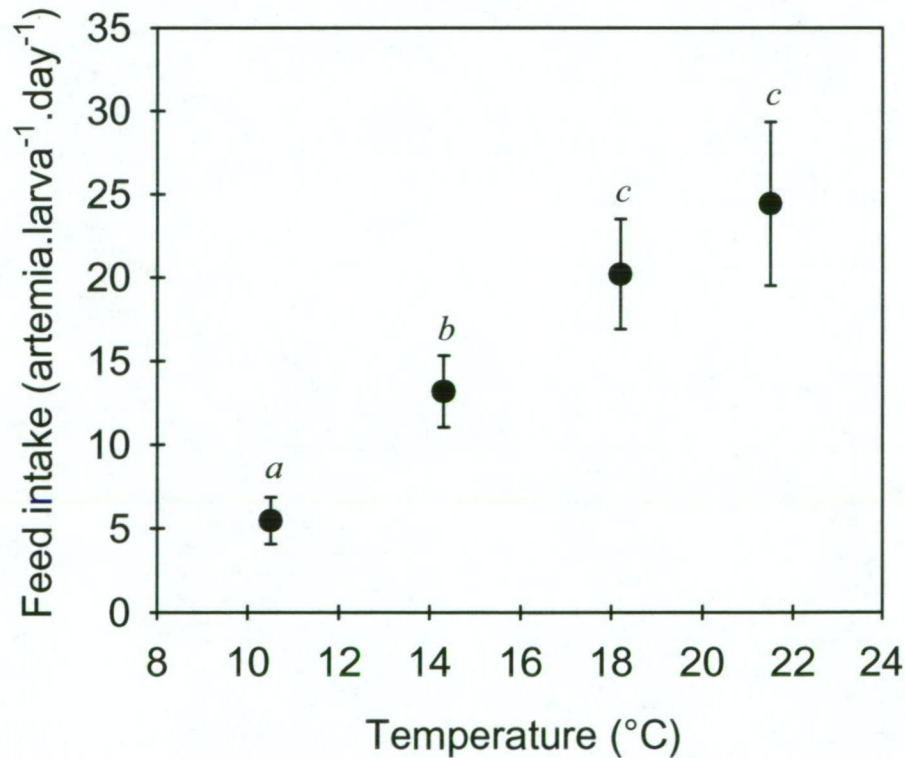


Figure 2.6 Mean (\pm SD) daily consumption of *Artemia* nauplii by mid-stage I *Jasus edwardsii* larvae reared at different temperatures. Treatments sharing the same letter were not significantly different (N-K, $P>0.05$).

2.4.2 *Photoperiod trial*

2.4.2.1 *Survival and cannibalism*

There was no effect of photoperiod on survival from stage I to stage III (PT, $F_{4,20} = 0.99$, $P = 0.436$), and although survival significantly declined with time (F stat., $F_{2,40} = 62.88$, $P < 0.0001$), mortality was not linked to photoperiod during development (F stat., $F_{8,40} = 1.07$, $P = 0.403$; Table 2.2). There was no significant effect of photoperiod on the mortality due to cannibalism at stage II and III (ANOVA, $F_{4,20} = 0.61$, $F = 0.658$; Fig. 2.7).

2.4.2.2 *Rate of development and growth*

Photoperiod had a significant effect on the intermoult period (PT, $F_{4,20} = 3.42$, $P < 0.05$) during the developmental period studied. While the duration of the intermoult period increased significantly during development (F stat., $F_{2,40} = 167.48$, $P < 0.0001$; Fig. 2.8), there were significant ontogenic variations in response to photoperiod (F stat. on stage \times photoperiod, $F_{8,40} = 5.79$, $P < 0.0001$). Changes in the intermoult duration response of phyllosoma to photoperiod occurred from stage I to stage II (F stat., $F_{4,20} = 5.43$, $P < 0.01$), and from stage II to stage III (F stat., $F_{4,20} = 3.49$, $P < 0.05$). Indeed, the absence of a treatment effect at stage I (ANOVA, $F_{4,20} = 1.92$, $F = 0.147$) was followed, at stage II, by a decrease in intermoult duration as daily light exposure increased (ANOVA, $F_{4,20} = 6.52$, $P < 0.01$; Fig. 2.8). The effect of photoperiod on the intermoult duration of stage III larvae (ANOVA, $F_{4,20} = 4.27$, $P < 0.05$) was again different with larvae exposed to 24L becoming significantly slower to develop than larvae reared under 6L, 12L and 18L (LSD, $P < 0.05$; Fig. 2.8).

There was no statistically detectable overall effect of photoperiod on moult increment (PT, $F_{4,20} = 2.69$, $P = 0.061$) during growth from stage I to stage III. Moult increment did not change with developmental stage (G-G univ., $F_{1,56,31.29} = 0.18$, $P = 0.781$) and this was consistent across all treatments (G-G univ., $F_{6,26,31.29} = 2.35$, $P = 0.053$; Fig. 2.9). However, there was a subtle developmental change in response to photoperiod from stage II to stage III (PT, $F_{4,20} = 2.94$, $P < 0.05$). In

contrast with the two previous stages, stage III larvae showed a marked response to photoperiod (ANOVA, $F_{4,20} = 2.95$, $P < 0.05$) and the ontogenic change in response observed at this stage was due primarily to a significant (LSD, $P < 0.05$) decline in growth in larvae reared in 24L compared to larvae reared under 6L, 12L and 18L (Fig. 2.9).

2.4.2.3 Feed consumption

There was a significant overall effect of photoperiod on feed consumption in early-stage phyllosoma (PT, $F_{4,20} = 4.09$, $P < 0.05$). Feed intake increased significantly during development (F stat., $F_{2,40} = 77.59$, $P < 0.0001$; Fig. 2.10) but this increase was influenced by photoperiod (F stat., $F_{8,40} = 2.21$, $P < 0.05$). At stage I increased Artemia consumption was associated with the lengthening of the light phase (ANOVA, $F_{4,20} = 4.07$, $P < 0.05$; Fig. 2.10). Although there was no significant effect of photoperiod on the feed intake of stage II larvae (ANOVA, $F_{4,20} = 2.35$, $P = 0.090$), the trend of response was consistent with observations made in stage I larvae (F stat., $F_{4,20} = 1.09$, $P = 0.389$). The feeding pattern shifted significantly from state I to stage III (F stat., $F_{4,20} = 4.64$, $P < 0.01$) due to a relative, although marginal, decline in feed intake in larvae reared under 18L and 24L. However, this shift in response at stage III did not result in a marked effect of photoperiod at this stage (ANOVA, $F_{4,20} = 2.83$, $P = 0.052$).

Table 2.2 Mean (\pm SD) percent survival to stage II, III and IV in *Jasus edwardsii* larvae reared from hatch under different photoperiods.

Survival to stage	Photoperiod (hours of light day ⁻¹)				
	0	6	12	18	24
II	97.14 \pm 6.39	97.14 \pm 2.61	97.14 \pm 2.61	99.00 \pm 2.24	99.13 \pm 1.94
III	89.52 \pm 6.21	88.61 \pm 7.27	89.52 \pm 9.16	88.52 \pm 4.17	93.75 \pm 4.89
IV	76.19 \pm 4.76	83.90 \pm 5.56	86.67 \pm 8.52	83.67 \pm 5.38	89.23 \pm 6.35

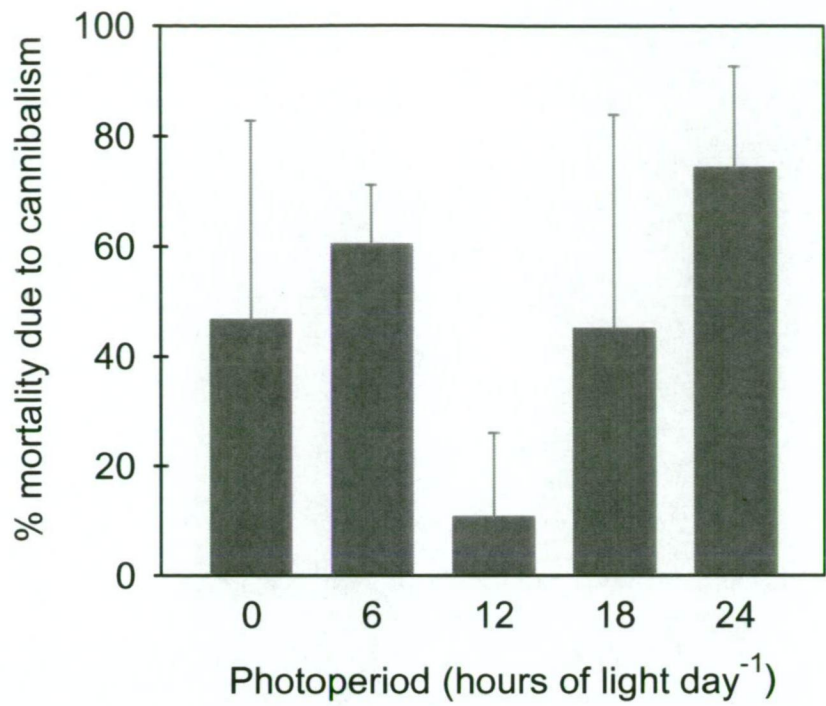


Figure 2.2 Mean (\pm SD) mortality due to cannibalism in stage II and III *Jasus edwardsii* larvae reared under different photoperiods.

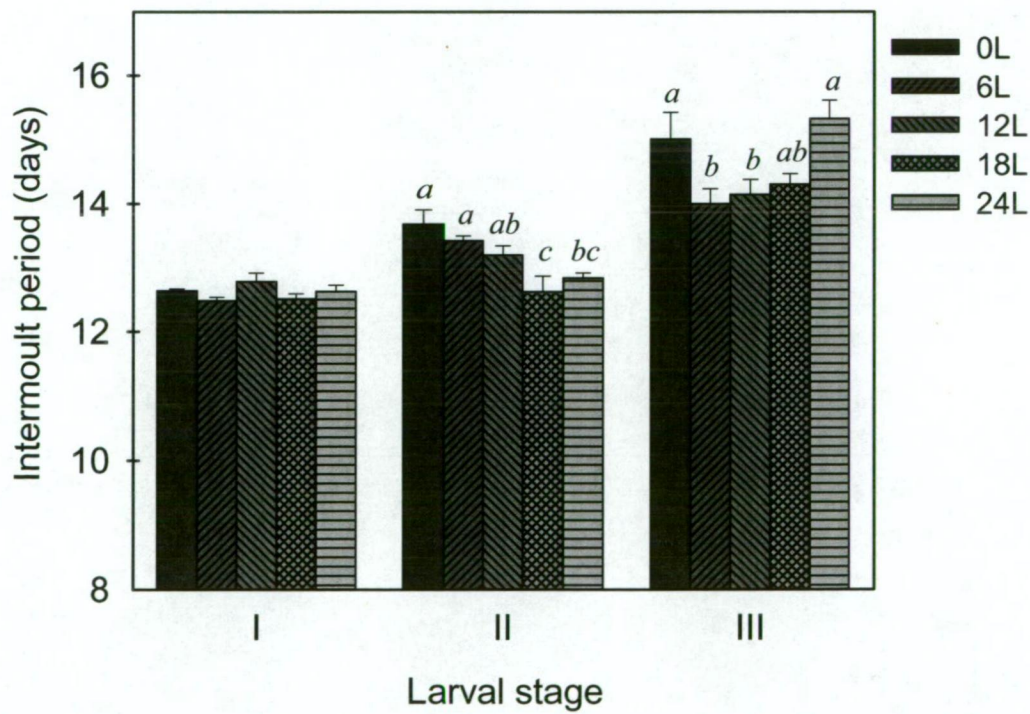


Figure 2.3 Mean (\pm SE) intermoult period in the first three stages of the larval development of *Jasus edwardsii* during rearing at different photoperiods. Treatments within stage sharing common letters were not significantly different (LSD, $P > 0.05$).

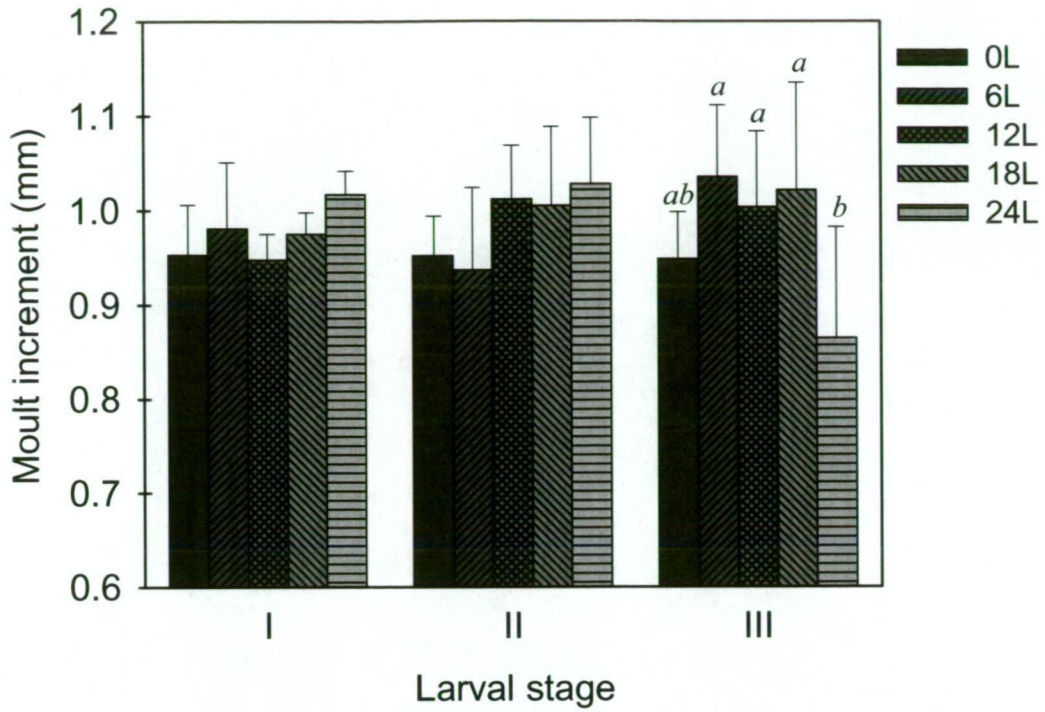


Figure 2.4 Mean (\pm SE) moult increment in the first three stages of the larval development of *Jasus edwardsii* during rearing at different photoperiods. Treatments within stage sharing common letters were not significantly different (LSD, $P>0.05$).

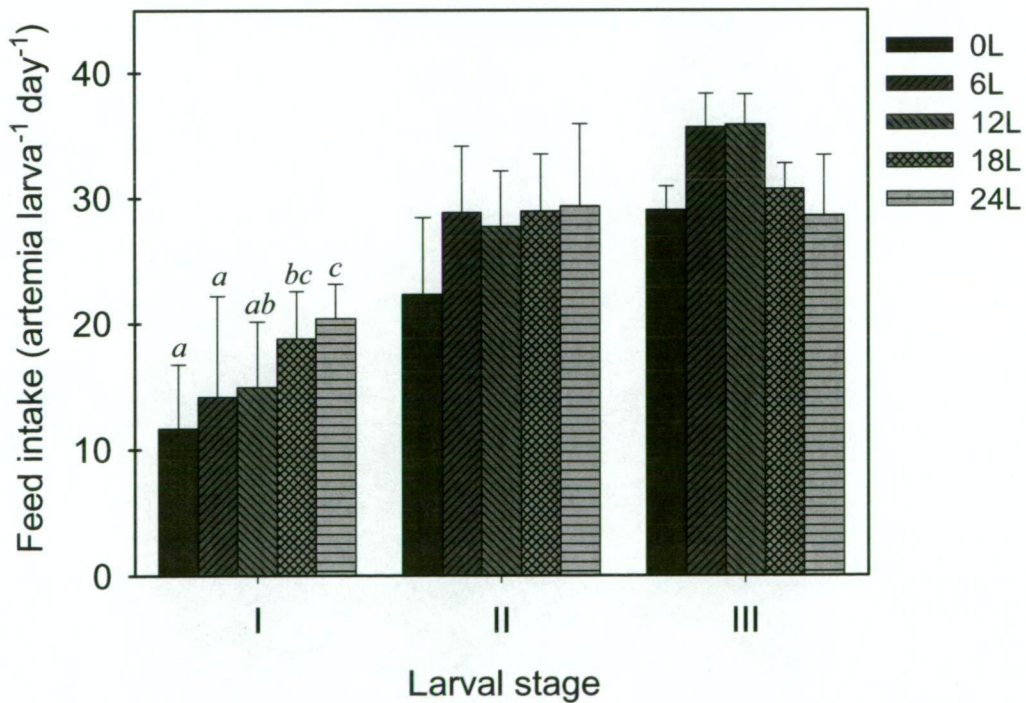


Figure 2.5 Mean (\pm SD) feed consumption in the first three stages of the larval development of *Jasus edwardsii* during rearing at different photoperiods. Treatments within stage sharing common letters were not significantly different (LSD, $P>0.05$).

2.5 Discussion

The effect of temperature and photoperiod on the survival, rate of development and growth throughout the first three larval stages of *J. edwardsii* was investigated in groups of larvae reared in static water systems. In contrast, Moss *et al.* (1999) and Tong *et al.* (2000b) working on the same species, reared larvae individually in a turbulent upwelling environment to study aspects of temperature and light intensity. There is some discrepancy between the results of their work and those presented here, and throughout the discussion, further reference will be made to the differences in experimental approach between the present and past studies.

2.5.1 Temperature

The survival of decapod larvae (and poikilotherms in general) commonly declines at the lower and upper extremes of a species specific temperature tolerance range. Likewise, high mortality was experienced post-moult into stage II in *J. edwardsii* larvae reared at 21.5°C, the high extreme of the experimental range. Antibiotics were used to prevent diseases that may be caused by the proliferation of opportunistic bacteria. In addition, the bacteriological analysis suggested a normal bacterial flora (Appendix III) in moribund larvae collected in the 21.5°C and 18.2°C treatments. Therefore, possible causes of mortality at high temperature include: (1) the burden of an energetic imbalance at moult from stage I to stage II; (2) oxygen starvation at moulting; (3) osmotic stress at higher salinity caused by evaporation (unfortunately, salinity levels were not monitored, however, there were obvious signs of evaporation at 21.5°C); (4) the disruption of cellular membrane structure and functions (Willmer *et al.*, 2000); (5) or a combination of these factors around the time of moult. The high mortality reported by Tong *et al.* (2000b) in *J. edwardsii* larvae reared at 12°C is not consistent with the high survival achieved at 10.5°C in the present study. Possible causes for discrepancy of outcome between the two studies are discussed later.

The rate of development of poikilotherms is closely linked to temperature. The relationship between the intermoult duration in stage I *J. edwardsii* and temperature between 10.5°C and 21.5°C was curvilinear as described by the Bělehrádek's expression. Similar patterns of response were reported in *Pandalus jordani* (Rothlisberg, 1979), *Cancer irroratus* (Johns, 1981), *Ranina ranina* (Minagawa, 1990), *Panulirus japonicus* (Matsuda and Yamakawa, 1997) and *Lithodes aequispinus* (Paul and Paul, 1999). The Bělehrádek's function indicated a theoretical biological zero of 9.4°C for larval development at stage I. Tong *et al.* (2000a) found a lower theoretical biological zero of 7.53°C for embryonic development in *J. edwardsii*. However, these two figures are hardly comparable, firstly because they relate to a different developmental phase (endogenous feeding embryos compared to exogenous feeding larvae), and secondly, because Tong *et al.* (2000a) used linear extrapolation to determine the biological zero for embryonic development as opposed to the Bělehrádek's expression applied here.

The duration of the intermoult period progressively increased throughout the early stages at both 14.3°C and 18.2°C. Previous observations on the rate of development of *J. edwardsii* larvae reared in an upwelling culture system indicated little increase in the duration of the intermoult period until the fifth stage (Illingworth *et al.*, 1997). This suggests either that static systems are less than optimum for the long term rearing of phyllosomas, possibly because of water quality deterioration, or that larvae underwent a shift in environmental requirements (other than temperature) with ontogenic development. Continuous lighting may have contributed to the progressive extension of the intermoult period observed in the present study since there was a marked delay in development in stage III larvae reared under constant light in the photoperiod experiment.

At stage I, maximum body growth was found at 18.2°C compared to animals reared at 10.5 and 21.5°C. Similar larval growth patterns were found in other crustacean species with smaller individuals obtained at the cool and warm extremes (Rothlisberg, 1979; Johns, 1981; Minagawa, 1990; Kumlu *et al.*, 2000; see also review by Anger, 2001). Sweeney and Vannote (1978) suggested that the smaller arthropod larvae obtained under non-optimum regimes (i.e. cooler or

warmer) are the result of a disequilibrium between the timing of development (e.g. ecdysis) and larval growth (e.g. energy storage). If this is the case in *J. edwardsii*, larvae hatching in northern Tasmanian waters between November and December (e.g. King Island; Winstanley, 1977) at sea surface temperatures ranging from 12.7°C to 17.4°C (records from 1989 to 1999, King Island ⁽¹⁾) are likely to grow larger and be subsequently fitter (Hare and Cowen, 1997; see also Appendix IV) than their counterparts hatched from July to October in southern Tasmanian waters at temperatures ranging from 8.6°C to 14.1°C ⁽¹⁾ (Maatsuyker Island). This hypothesis would not hold true if northern and southern larval populations had different temperature optima for growth. In addition, food availability and/or accessibility would also be expected to have a significant effect on the growth processes. Finally, the assumption of size dependence fitness in *J. edwardsii* larvae needs further investigated in terms of predator avoidance, predatory efficiency, and post-metamorphosis performance among other criteria. Subsequent growth observed in the present study at 14.3 and 18.2°C followed a similar pattern to intermoult period with moult increment being overall larger at 18.2°C than at 14.3°C, and moult increment declining independently of temperature throughout development.

Daily feed consumption increased from 10.5 to 18.2°C but did not significantly increase thereafter to 21.5°C. Considering that larvae at 21.5°C would have a higher metabolic rate than larvae at 18.2°C, a relative decline in feed intake at elevated temperatures may result in an energy imbalance. This shortfall in energy intake in concomitance with the disequilibrium between the timing of development and larval growth suggested by Sweeney and Vannote (1978), could explain the sharp decline in larval growth observed at stage I from 18.2 to 21.5°C as opposed to the steady increase in moult increment seen between 10.5 and 18.2°C. Tong *et al.* (2000b), also working with stage I *J. edwardsii* larvae hatched from embryos incubated at ambient temperature and at 15°C, reported on a similar pattern of increased feed consumption from 15 to 18°C which then steadied between 18°C and 24°C. However, these authors did not find any difference in growth for larvae reared between 18 and 24°C. Differences in the methodological approach between the present study and work done in New

⁽¹⁾ NOAA satellite AVHRR imagery.

Zealand could explain the discrepancy in results reported here. The rearing of individual animals in an upwelling system (Tong *et al.*, 2000b) contrasts in many aspects with the group rearing in static water used here. Aspects of the culture conditions that could have influenced growth include: (1) animal density (i.e. individual vs. group rearing); and (2) turbulence (i.e. upwelling vs. static water). In *Palaemon serratus* (Reeve, 1969) and *Carcinus maenas* (Dawirs, 1982), larvae reared in isolation grew to a larger size than animals reared communally. Turbulence was reported to play an important role in the feeding of a number of zooplanktonic species by raising the encounter rate between predators and preys (Dower *et al.*, 1997). Therefore, the experimental approach used by Tong *et al.* (2000b) is likely to have stimulated feeding and in turn reduced the energy deficiency resulting from the group rearing of larvae at 21.5°C in the present study.

Under the conditions of this experiment, a temperature of around 18°C was suitable for the rearing of the first three larval stages in *J. edwardsii*. Further work is now required to define temperatures required for the culture of mid and late stage animals as temperature preferences may change with development (e.g. *Panulirus japonicus*; Matsuda and Yamakawa, 1997).

2.5.2 Photoperiod

Even though the literature shows a similar pattern across species in the response of crustacean larvae (and of poikilotherms in general) to temperature (Anger, 2001), there is no obvious inter-species consistency in their response to photoperiod. Variation in the length of the light phase can influence survival (e.g. *Homarus americanus*; Templeman, 1936), rate of development (e.g. *Carcinus maenas*; Dawirs, 1982) and growth (e.g. *Thenus orientalis*; Mikami, 1997) of decapod larvae. Nevertheless, other species such as *Sesarma reticulatum* (Costlow and Bookhout, 1962), *Palaemonetes vulgaris* (Knowlton, 1974) and *Pandalus borealis* (Wienberg, 1982) were reported to be unaffected by photoperiod.

While the survival of *J. edwardsii* larvae was high across treatments throughout the first three stages of development, photoperiod affected intermoult period and growth with significant ontogenic differences in response observed at each of the three stages investigated. Gardner and Maguire (1998) reported on the increased incidence of cannibalism in *Pseudocarcinus gigas* zoeas as photoperiod extended. In the present study, the mortality due to cannibalism among *J. edwardsii* larvae was highly variable (i.e. 11 to 74%) across photoperiod treatments and did not appear to be related to the length of the light phase, or to feeding activity under the various light regimes tested.

A number of species including *Carcinus maenas* (Dawirs, 1982) and *Thenus orientalis* (Mikami, 1997) showed consistent growth response to photoperiod throughout larval development. This contrasts with the changes in photoperiod requirement observed in the early stages of larval development in *J. edwardsii*. At stage II, shorter intermoult periods were achieved with increasing photoperiod, while at stage III, larvae reared in light/dark treatments grew comparatively larger and in less time than larvae reared under complete darkness and constant light. Mikami (1997), suggested that the slow growth observed in *Thenus orientalis* larvae reared under constant light could be related to an inability to feed by positively phototactic animals drawn away from the food sitting at the bottom of the culture vessel. Shallow (~2cm deep) bowls and diffuse lighting were used in the present study to avoid such artefacts. Therefore, the stress from constant exposure to light is likely to have been solely responsible for the relative decline in feed intake observed in stage III larvae reared under 24L. Suppressed growth caused by reduced food rations was clearly demonstrated in *J. edwardsii* larvae by Tong *et al.* (1997). Furthermore, the hypothesis that reduced growth under sub-optimum conditions is caused by a disequilibrium between the timing of development and energy storage (Sweeney and Vannote, 1978) may also applies to photoperiod since declining growth appears to be associated with reduced feed intake in *J. edwardsii* larvae (e.g. stage II and III under 0L, and stage III under 24L).

Similar to the reduced larval development reported in *Callinectes sapidus* (Sandoz and Rogers, 1944) and *Carcinus maenas* (Dawirs, 1982) reared in the

dark, *J. edwardsii* larvae cultured in the present study performed poorly in complete darkness in terms of rate of development (i.e. at stage II and III), growth (i.e. at stage III) and feed intake (i.e. at stage I). In contrast, Moss *et al.* (1999) working on the same species, found that although stage I larvae fed less in the dark, they grew to a larger size than larvae reared under constant high light intensities (0.1 and 10 $\mu\text{mol s}^{-1} \text{m}^{-2}$). Once again, differences in experimental approach (isolation vs. group rearing and turbulent vs. static environment), could account for the discrepancy in the results obtained between these authors and the present study.

The similar feed consumption observed in a turbulent environment between larvae reared in complete darkness and individuals under constant light (Moss *et al.*, 1999) suggests that *J. edwardsii* phyllosomas are not visual feeders but capture preys from random encounter. In this case, the low feed intake presently observed in larvae reared in static water in the dark would be the result of a reduced level of activity. In *Callinectes sapidus* larvae, McConaughy *et al.* (1991) have associated a higher night-time feeding with the 60% increase in swimming speed at night reported by Sulkin *et al.* (1979). Since light stimulates swimming in phyllosoma of the Palinuridae and Scyllaridae families (Ritz, 1972; Mikami, 1995; Chapter 3, Fig. 3.8 and Table 3.9), a lack of light would cause a reduction in activity and a drop in encounter rate between larvae and preys.

Work by Mikami (1997) indicated that a light/dark phase was necessary to synchronise moulting of *Thenus orientalis* larvae around dawn. Rhythmic environmental factors are also known to regulate internal physiological processes (Sulkin *et al.*, 1979), and Aiken (1969) suggested that photoperiod affects the ecdysial rhythm in crustaceans. These are possible explanations for the stimulated rate of development and growth observed in stage III *J. edwardsii* larvae reared under light/dark regimes.

In view of the results obtained in this trial, a continuous light regime is suitable for rearing *J. edwardsii* larvae through stages I and II as it stimulates feeding. However, stage III larvae appeared to require a light/dark regime, which enhanced their rate of development and growth.

2.6 Conclusions

The methodology used by Moss *et al.* (1999) and Tong *et al.* (2000b) to study the effect of photoperiod and temperature exposes individual larvae to the turbulent environment normally experienced in a tank. As such, this approach can be deemed a more suitable assessment tool to determine environmental culture conditions for *J. edwardsii* larvae than the group rearing in static water applied in the present study. However, from the perspective of defining culture conditions for mass rearing, experimental work conducted on individual larvae excludes the animal interaction that would normally take place within a culture vessel. Phyllosomas occur at low density in their oceanic habitat (Bruce *et al.*, 2000), and animal interaction at relatively high densities could be a significant factor to take into account when defining environmental preferences for propagation in a hatchery situation. Therefore, the use of a system combining both water mixing and group rearing could be a suitable alternative to both methods for experimental work on propagation techniques in *J. edwardsii*.

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Chapter 3

The ontogeny of
behavioural and physiological
responses to
temperature and light intensity
throughout
early larval development

3.1 Abstract

The behavioural (swimming speed, photoresponse, phototaxis and geotaxis) and physiological (oxygen consumption, nitrogen excretion and feed intake) responses to temperature and light intensity were examined in early-stage *Jasus edwardsii* larvae sampled from mass culture tanks at successive stages. Phyllosoma were photokinetic and positively phototactic at hatching and became predominantly negatively phototactic by day 2. At mid-stage I however, larvae were evenly distributed between positive and negative photoresponse under the lowest light intensity tested ($2.9 \cdot 10^{14}$ quanta $\text{s}^{-1} \text{cm}^{-2}$). This response was not observed again at mid-stage II or mid-stage III, implying that older larvae may have a lower light threshold for reversal of phototaxis. The level of illuminance might therefore be an important consideration for controlling behaviour in culture. Rising temperature caused a gradual decline in the negative geotaxis of newly-hatched larvae. Except for signs of avoidance of cold water in mid-stage I larvae, the response to temperature faded with age and larvae were predominantly positively geotactic at mid-stage II from 10 to 22°C. This indicated that other factors would influence depth regulation at this stage. The physiological response observed in larvae exposed to conditions ranging from 10 to 22°C was characterised by a relative decline in weight specific oxygen consumption at elevated temperatures. While feed intake followed the same trend in stage I and II larvae, nitrogen excretion increased linearly within the same temperature range. Therefore, high temperatures (~22°C) may cause an energetic imbalance and reduce growth potential in early stages. The physiological response of larvae from hatching to stage V was higher under light than in the dark. This was primarily attributed to the increase locomotor activity associated with photoresponse. Light ranging in intensity from $7.7 \cdot 10^{12}$ to $3.9 \cdot 10^{14}$ quanta $\text{s}^{-1} \text{cm}^{-2}$ is recommended to stimulate feeding in early larval stages and may be followed by a resting dark phase to maximise feed assimilation.

3.2 Introduction

Temperature and light intensity are two of the most variable components of the abiotic environment of zooplankton. While both factors are depth dependent, light intensity also varies on a diurnal basis. At sea, *Jasus edwardsii* larvae are distributed over a wide depth profile both during the day and at night (Booth, 1994; Bruce *et al.*, 2000). Behavioural studies (Russell, 1925; Boden and Kampa, 1967; Harding *et al.*, 1987; Haney, 1988; among others) indicated that the majority of zooplankters undergo vertical migration within their respective habitat. There is evidence from field surveys that *J. edwardsii* larvae are no exception to this behavioural trait of crustacean zooplankton (Booth, 1994; Bruce *et al.*, 2000). Diel vertical migration is thought to play an important role in predator avoidance (Gliwicz and Pijanowska, 1988) and advection transport (Phillips and McWilliam, 1986). In addition, McLaren (1963) proposed that the variations in temperature encountered by migrating animals was of significant metabolic advantage since it allowed them to feed actively in warm surface waters before sinking to colder strata where their food assimilation and growth would be maximised. However, Haney (1988) noted later that there was little experimental evidence to support the possible metabolic advantage of depth regulation in zooplankton. In other words, there is no evidence for differences in thermal optima between different metabolic functions.

From an aquaculture perspective it is essential to define the influence of temperature and light intensity on the behaviour and physiology of *J. edwardsii* larvae. The behavioural response of many decapod larvae to temperature and light has already been the focus of extensive work and was reviewed by Sulkin (1984) for brachyuran larvae. There are many similarities in the response of decapod larvae to temperature and light and these will be discussed later in view of the results obtained in the present study. Generally however, the response is species dependent and reflects environmental preferences, and also the environmental history of the experimental animals (Forward, 1974; Stearns and Forward, 1984). From a physiological perspective, information on behavioural

responses is crucial to the understanding of changes in metabolism driven by shifts in locomotor activity under variable environmental conditions. The physiological response of animals to abiotic factors can assist the aquaculturist in understanding the processes underlying development and growth of cultured animals. While the effect of temperature on the physiology of decapod larvae is well documented, little information is available on the effect of light intensity on physiological processes such as oxygen consumption and nitrogen excretion. The series of experiments described in this chapter aimed at defining the behavioural and physiological responses of early-stage *J. edwardsii* larvae to temperature and light intensity in an effort to understand but also predict the effect of these factors on larval development. The methods used here were adapted from experimental procedures available throughout the vast scientific literature that deals with the present topics of interest.

3.3 Material and methods

3.3.1 *Larvae*

In the following trials, larvae were examined at various stages of development from directly after hatching (newly-hatched) to stage V (stages described by Lesser, 1978). Larvae were obtained from ovigerous females caught in coastal waters around Tasmania and kept in captivity at the TAFI Marine Research Laboratories (Australia). Newly-hatched larvae were collected from hatching tanks during September-November of 1999 and 2000 at ambient temperatures ranging from 12.0 to 17.1°C. Larvae from 15 females were reared through to stage V at 18°C following the method described by Ritar (2001). Each brood was reared in two 10 L flow-through tanks, each initially stocked with 1000 newly-hatched phyllosomas. The density gradually declined as larvae were sampled for experiments at mid-stages I, II, III and V (see Table 3.1 for age and morphometrics). During mass culture phyllosomas were fed adult *Artemia* (1.5-3 mm) at a ration of 180 mg of dry weight *Artemia* per 10 L of water, and uneaten food and dead larvae were removed daily. The tanks were exchanged and cleaned weekly. To avoid interference from possible circadian behavioural patterns, all experiments were conducted between 1000 and 1600 h. In all temperature trials, larvae (except newly-hatched animals) were acclimated to their experimental temperature for 24 h prior to the start of an experiment.

3.3.2 *Behaviour*

3.3.2.1 *Temperature*

The geotactic response was examined at seven temperatures ranging from 10.3 to 21.9°C in newly-hatched *J. edwardsii* larvae, and at mid-stages I and II. Seven sets of three glass columns (300 x 100 x 100 mm) with sea water were maintained at different temperatures inside thermo-regulated aquaria. Larvae were introduced to each column and left to acclimate in the dark for 1 h before

observation of geotaxis. Observations were made on 20 individuals per column in newly-hatched and mid-stage I larvae and in 16 animals per column at mid-stage II. Three broods were tested, one per column within each temperature group. The position of the larvae in the column (upper or lower half) was recorded using a hand held torch. Since *J. edwardsii* larvae are phototactic, observations were only made in one half of the columns starting from the middle. Data were analysed with repeated measures ANOVA following arsine square root transformation to meet the assumption of homoscedasticity.

3.3.2.2 Light

The effect of light intensity on the locomotor activity of phyllosoma was examined in newly-hatched larvae swimming towards a light source (20 watt quartz halogen) adjusted to low ($2.9 \cdot 10^{14}$ quanta $\text{s}^{-1} \text{cm}^{-2}$), medium ($2.5 \cdot 10^{15}$ quanta $\text{s}^{-1} \text{cm}^{-2}$), or high ($1.8 \cdot 10^{16}$ quanta $\text{s}^{-1} \text{cm}^{-2}$) light intensity as measured at the middle of the experimental column (Fig. 3.1) with a Biospherical QSL 100 light meter. The different light intensities were obtained by placing layers of aluminium fly screen and drawing paper in front of the light source. These filters did not affect the peak wavelength of the light source (652 nm) as measured with a portable spectroradiometer (Li-Cor, LI-1800). Conversions of light intensities from quanta $\text{s}^{-1} \text{cm}^{-2}$ to W m^{-2} and $\mu\text{mol s}^{-1} \text{m}^{-2}$ are presented in Appendix V. After the light was turned on, larvae were released from a small cage placed at the middle of the column (Fig. 3.1.A). Their swimming towards the light (Fig. 3.2) was recorded with an overhead camera connected to a video recorder. Swimming speed (cm s^{-1}) was later measured on a monitor as the time taken for larvae to cover a 10-cm horizontal section (distance between the cage and the 'finish line', see Fig. 3.2) of the chamber. A total of 20 larvae from each of four broods were examined at each light intensity. The effect of the log transformed light intensity on swimming speed was described with linear regression analysis.

The phototactic response of *J. edwardsii* larvae was examined throughout stage I at two-day intervals from hatching until day 10, and at mid-stage II and mid-stage III. Larvae in stock cultures started to moult into stage II at day 10, and only stage II phyllosomas (49-85% of the population in this age class) were

sampled on this day. The light regime (photoperiod and light intensity) was not rigorously controlled during mass culture and there may have been some differences of light intensity between tanks. Since previous light experience may affect the behavioural response of decapod larvae (Forward, 1974; Stearns and Forward, 1984), phyllosomas were placed in the dark for 2 h prior to each trial in an attempt to minimise tank effect. Larvae were introduced in the partitioned centre of the column (Fig. 3.1.B) and were given 10 min to recover from handling after which, the light source adjusted to $2.9 \cdot 10^{14}$, $2.5 \cdot 10^{15}$, or $1.8 \cdot 10^{16}$ quanta $\text{s}^{-1} \text{cm}^{-2}$ was switched on and the partitions removed. The partitions were replaced after 10 min of exposure to light and larvae were counted in each of the three sections of the column. The same protocol was repeated in the dark. For each age class, phototaxis was examined in 15-20 larvae from each of four different broods and at each light intensity. The photoresponse was analysed in terms of percent positively phototactic larvae of the responsive animals (i.e. larvae outside the centre section of the column). Repeated measures ANOVA on the arcsine square root transformed response rates was used to analyse the change in response to light intensity during stage I and between mid-stages I (i.e. day 6), II, and III. Developmental changes in response were further described within each age class by comparing the response of larvae subjected to light with the behaviour of larvae in the dark control (Dunnett's test).

3.3.3 *Physiology*

The following observations were conducted in relation to temperature in larvae grown to mid-stage I, II and III, and in newly-hatched, mid-stage I, II, III and V larvae under different light intensities. Oxygen consumption and nitrogen excretion were determined in two separate broods and due to number requirements for all experiments (i.e. behaviour and physiology x temperature and light intensity) and the occasional mortality event during mass culture, larvae from different broods were used at each stage. At mid-stage V, nitrogen excretion was measured in a single progeny. Feed intake was examined in 15-20 larvae from a minimum of three broods at each age class and under each environmental condition tested. In the temperature trials, physiological observations were

conducted in seven aquaria each equipped with a thermostat to maintain temperatures (mean \pm SD) of 9.9 ± 0.3 , 12.1 ± 0.2 , 14.0 ± 0.1 , 16.0 ± 0.1 , 18.0 ± 0.1 , 20.0 ± 0.1 and $21.8 \pm 0.1^\circ\text{C}$. Due to limitation in the number of phyllosomas available, the response of stage III larvae was only examined at 9.9 ± 0.3 , 14.0 ± 0.1 , 18.0 ± 0.1 , and $21.8 \pm 0.1^\circ\text{C}$. Light-proof chambers were constructed to compare the physiological response of larvae placed in the dark, and exposed to low ($7.7 \cdot 10^{12}$ quanta $\text{s}^{-1} \text{cm}^{-2}$) and high ($3.9 \cdot 10^{14}$ quanta $\text{s}^{-1} \text{cm}^{-2}$) light intensities. In the light treatments, a quartz halogen light source was reflected on a white ceiling above the animals to provide even distribution of light throughout the chambers. Each light-proof chamber was fitted with a water bath maintained at an incubation temperature of 18°C .

3.3.3.1 Oxygen consumption (VO_2)

Two different respirometer designs were used to measure oxygen consumption in *J. edwardsii* larvae (Fig. 3.2). Plastic syringes (Fig 3.2.A) were used in the temperature experiment while transparent glass respirometers (Fig. 3.2.B) were preferred for testing the effect of light intensity. Animal density and period of exposure are outlined in Table 3.2. The respirometers were filled with UV sterilised, $0.2 \mu\text{m}$ filtered sea water treated with oxytetracycline (25 ppm) to minimise background microbial respiration. Larvae were left to recover from handling stress while acclimating to the respirometers for 1-2 h before a first water sample (0.75 ml) was drawn to determine the initial oxygen saturation level. The oxygen content was left to decline in the respirometer (see incubation period in Table 3.2) and a second water sample was taken to determine final oxygen tension. To avoid oxygen stratification, respirometers were gently shaken before each water sampling. Percent oxygen saturation of initial and final samples was measured with a polarographic electrode connected to a digital controller (Rank Brothers Ltd., UK). In each treatment, VO_2 was assessed in five replicate respirometers and two controls without phyllosoma. The dry weight (DW) of test animals was determined from three samples of larvae (from 10 individuals per sample in newly-hatched larvae to 2 individuals per sample at stage V) rinsed in a 0.9% solution of ammonium formate and dried for 24 h at 60°C . The weight of

each sample was measurement to the nearest 10 μg on a precision balance (Mettler AT261 DeltaRange, Mettler-Toledo AG, Switzerland). Oxygen consumption was expressed in $\mu\text{l O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$ after deduction of background respiration obtained from the control respirometers. Oxygen saturation in the respirometers was kept above 80% (Ikeda *et al.*, 2000) throughout these trials. However, dissolved oxygen fell below this level on one occasion (temperature experiment: stage II larvae from one brood incubated at 21.8°C) when final oxygen tension (mean $\pm\text{SD}$, $n = 5$) reached $3.8 \pm 0.2 \text{ ml O}_2 \text{ l}^{-1}$. This concentration was nonetheless near the P_{crit} determined for stage I larvae at elevated temperature ($3.9 \text{ ml O}_2 \text{ l}^{-1}$; see Chapter 5). The response to temperature at each stage and the ontogenic change in response at each light intensity were modelled according to the procedure described later in this section. Curves were compared with the Kimura Likelihood Ratio test.

3.3.3.2 Nitrogen excretion

Nitrogen excretion rates were determined in groups of larvae placed in acid cleaned, sealed glass vials containing UV sterilised, $0.2 \mu\text{m}$ filtered sea water treated with oxytetracycline (25 ppm). Details of the volume of the vials, number of animals per group and period of exposure to temperature or light intensity are presented in Table 3.2. At the end of the period of exposure, a 3-ml sample of water was taken from each of 5 replicate vials and 2 controls for each treatment. Ammonia concentrations were determined according to the method described by Solorzano (1969). Larval dry weight (DW) was obtained following the procedure outlined earlier, and excretion was expressed in $\mu\text{g NH}_4\text{-N mg DW}^{-1} \text{ h}^{-1}$ after the deduction of $\text{NH}_4\text{-N}$ concentration in the control vials. The effect of temperature on nitrogen excretion through development was assessed with analysis of covariance (ANCOVA) for lines with the same slope (Sokal and Rohlf, 1995). The effect of light intensity on nitrogen excretion was analysed with the Kimura Likelihood Ratio test after modelling the response through time at each intensity.

3.3.3.3 Feed intake

The effect of temperature and light intensity on the consumption rate of adult *Artemia* by phyllosoma was assessed in clear plastic jars. Experimental procedures are further detailed in Table 3.2. Note that larger preys were offered to stage V larvae. An average *Artemia* length was determined from the measurement of 20 *Artemia* for each feeding trial. Weight specific consumption of dry weight *Artemia* ($\mu\text{g Artemia mg DW}^{-1} \text{ h}^{-1}$) was computed following the conversion of *Artemia* length into dry weight according to the exponential function determined by Evjemo and Olsen (1999) for *Artemia franciscana*:

$$\text{Artemia dry weight} = 0.751 e^{(1.22 L)}$$

where L is *Artemia* length (mm). Response modelling and the Kimura Likelihood Ratio were used to describe the effect of temperature and light intensity on larval feed intake throughout early development. These procedures are detailed later in this section.

3.3.4 Physiological indicators

3.3.4.1 van't Hoff's rule

The Q_{10} was computed using the following formula (Ikeda *et al.*, 2000):

$$Q_{10} = (k_2 / k_1)^{10 / (T_2 - T_1)}$$

where k_2 and k_1 are respiratory rates (in $\mu\text{l O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$) observed at temperatures T_2 and T_1 (in $^{\circ}\text{C}$), respectively. A Q_{10} of around 1 indicates that no change in metabolic rate has occurred within the thermal range considered while a Q_{10} of around 2 indicates a doubling of metabolism for every 10°C increment. Values of the Q_{10} were obtained at stage I, II and III for three temperature ranges: $10\text{--}14^{\circ}\text{C}$, $14\text{--}18^{\circ}\text{C}$ and $18\text{--}22^{\circ}\text{C}$. The Q_{10} values obtained at each stage were grouped by temperature range for statistical analysis. The effect of temperature on the change in Q_{10} in stage I-III phyllosoma was assessed with the Welch ANOVA for data with unequal variance followed by a Games-Howell ad-hoc test (G-H).

3.3.4.2 Convection requirement index (CRI)

The CRI was calculated as the quotient of mean feed intake and mean oxygen consumption (Newell and Branch, 1980) for each environmental condition examined and at each stage. The CRI is an indicator of metabolic feeding efficiency. Given the computation of the CRI in the present study, statistical analysis was not applicable. Therefore, only careful interpretations were drawn from analysis conducted on each of the components of the quotient (i.e. oxygen consumption and feed intake).

3.3.4.3 O:N ratio

In order to describe the effects of environmental variables on the type of metabolic substrate (i.e. protein and/or lipid) oxidised by phyllosoma the atomic ratio between oxygen consumed and nitrogen excreted was computed according to the following formula (Agard, 1999):

$$\text{O:N} = a / b \times 1.25$$

where *a* and *b* are the mean weight specific oxygen consumption and nitrogen excretion respectively, obtained for given age class and environmental condition. As for the CRI, the nature of the O:N ratio computed in the present study did not allow for statistical analysis. Therefore the interpretation of the data was made according to the statistically tested effect of environmental variables on the components of the quotient (i.e. oxygen consumption and nitrogen excretion).

3.3.5 Statistical procedures

This section describes the procedures for repeated measures ANOVA and for the Kimura Likelihood Ratio test used in this study. In order to improve the clarity of the reading throughout the 'Results' section, the type of analysis used and the effect tested for will be indicated for each data set.

3.3.5.1 Repeated measures ANOVA

The Univariate Repeated Measures (JMP, 1995) procedure was used to assess developmental changes in behavioural response to temperature and light

(geotaxis, response, phototaxis). The between subject (temperature or light treatments) effect was determined with the Pillai's Trace multivariate test (PT). According to the significance of the sphericity test, a univariate unadjusted test (standard F statistic procedure; F stat.) or the Geisser-Greenhouse univariate test (epsilon adjusted for degrees-of-freedom; G-G univ.) were used to examine the within treatment effect of ontogenic development. The above analyses were conducted in JMP 3.1 and SPSS 10.0.

3.3.5.2 Response modelling

For data pertaining to the assessment of the influence of temperature, the models fitted to the raw data described the effect of temperature at each stage, whereas models applied to the responses to light intensity described the change in response to light with age at each intensity. Comparisons of curves were used to test for either ontogenic changes in response to temperature or the effect of light intensity.

Two criteria were used to fit the best type of model possible to the raw data but also to provide models easy to interpret and compare with each other: the Akaike's Information Criterion (AIC; Haddon, 2001); and the coefficient of determination adjusted for degrees of freedom (R^2_{adj}). Note also that to be statistically comparable models for a type of physiological response have to be the same for either each stage over a temperature range, or each light intensity throughout development. Therefore, compromise was sometimes required between the two criteria in order to obtain models that would be representative of the data and would also be relevant to the analysis. AIC and R^2_{adj} were computed in SigmaPlot 6.0, JMP 3.1 and Microsoft Excel 97.

3.3.5.3 Comparing lines and curves

The Kimura Likelihood Ratio test (KLR) was used to test for overall differences between a group of curves (e.g. respiration versus temperature at each stage, respiration versus age at each light intensity), and for ad-hoc tests of the coefficients of the model to determine the source of discrepancy between curves. Generally, the intercept was used to compare the level of response between stages

(i.e. temperature trial) or between treatments (i.e. light intensity trial), while the other coefficients of a model were compared to seek changes in the shape of the curve between stages or treatments. Note, however, that the intercept was of little comparative value when curves were of a significantly different shape. In this case analyses of variance (i.e. ANOVA and Least Significant Difference test) were used to compare between responses grouped at the stage level (i.e. temperature trial) or at the treatment level (i.e. light intensity trial). When data did not meet assumption of ANOVA (i.e. normality and/or homoscedasticity), Kruskal-Wallis ANOVA on ranks (K-W) followed by the Games-Howell test for multiple comparison (G-H) were carried out with SPSS 10.0 statistical software. The KLR analyses were carried out following the Microsoft Excel spreadsheet procedure described by Haddon (2001). Further details of the parameters tested in each model are given in the 'Results' section.

Table 3.2 Age, mean (\pm SE) length, and mean (\pm SE) dry weight of *Jasus edwardsii* larvae examined for behavioural and physiological response to temperature and light intensity.

Stage of development	Age (days)	Length (mm)	Dry weight (μ g)
newly-hatched	0	—	68.93 \pm 4.60
mid-stage I	4-6	2.02 \pm 0.03	128.57 \pm 4.84
mid-stage II	15-18	2.91 \pm 0.03	274.71 \pm 7.72
mid-stage III	25-30	3.80 \pm 0.04	501.57 \pm 24.09
mid-stage IV	52-55	5.90 \pm 0.13	1234.75 \pm 120.16

Table 3.3 Protocols used to determine oxygen consumption, nitrogen excretion and feed intake in *Jasus edwardsii* larvae.

	Stage			
	I	II	III	V
Oxygen consumption				
Volume of respirometer (ml)	12	12	12	17
Incubation period (h)			4-6	
Animals per respirometer (n)	8-10	4-5	3	2
Nitrogen excretion				
Volume of vial (ml)	9.5	9.5	9.5	25
Incubation period (h)			8-9	
Animals per vial (n)	8-10	5	3	4
Feed intake per larva				
Volume in jar (ml)	15	20	20	30
Incubation period (h)			24	
Initial Artemia in jar (n)	15	20	20	15
Size of Artemia (mm)	1.2-2.0	1.2-2.0	1.2-2.0	2.2-3.0

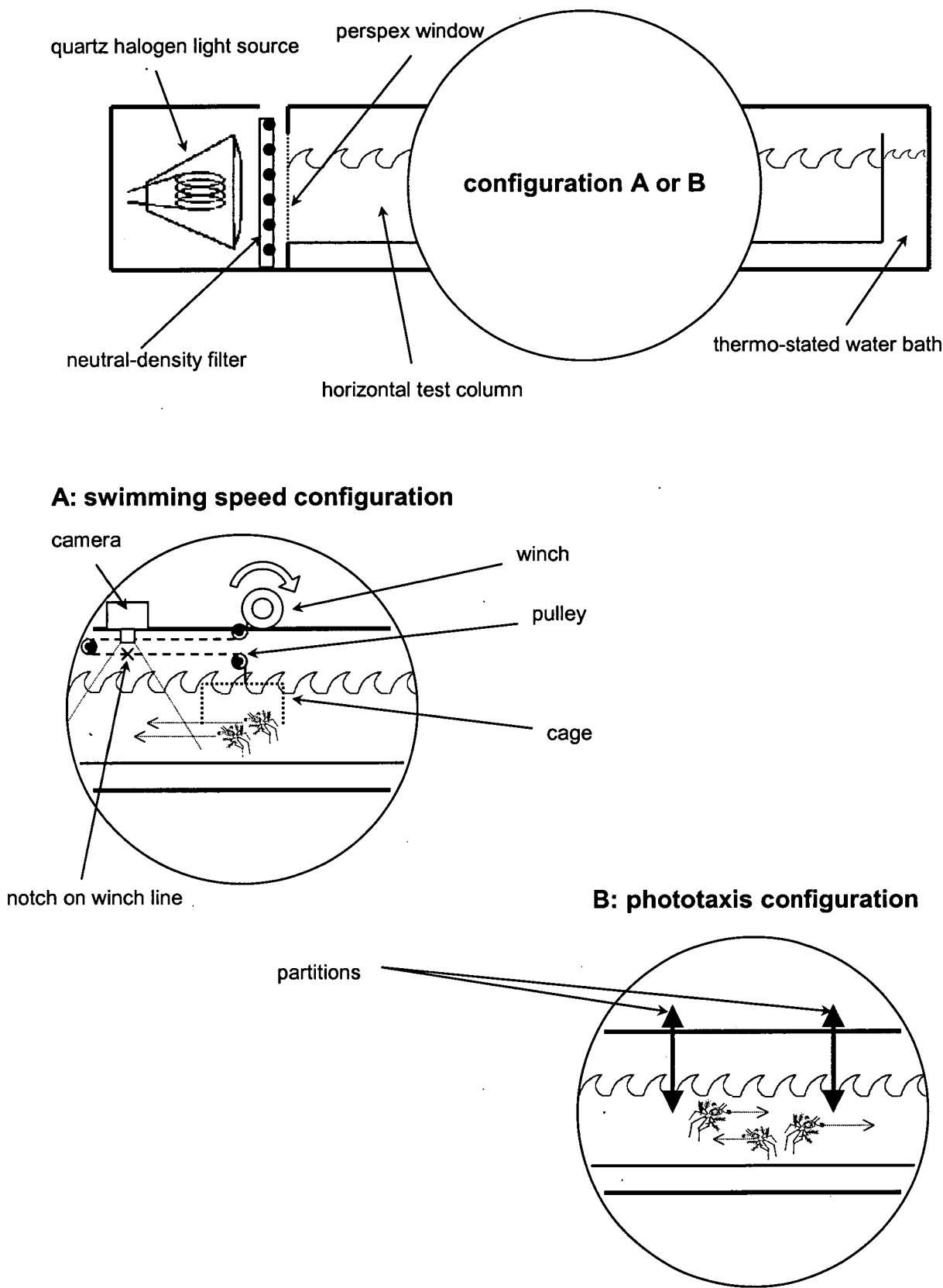


Figure 3.2 Schematic view of the apparatus used to test to swimming speed of newly-hatched *Jasus edwardsii* larvae (configuration A) and the phototaxis of phyllosoma from newly-hatched to stage III (configuration B).

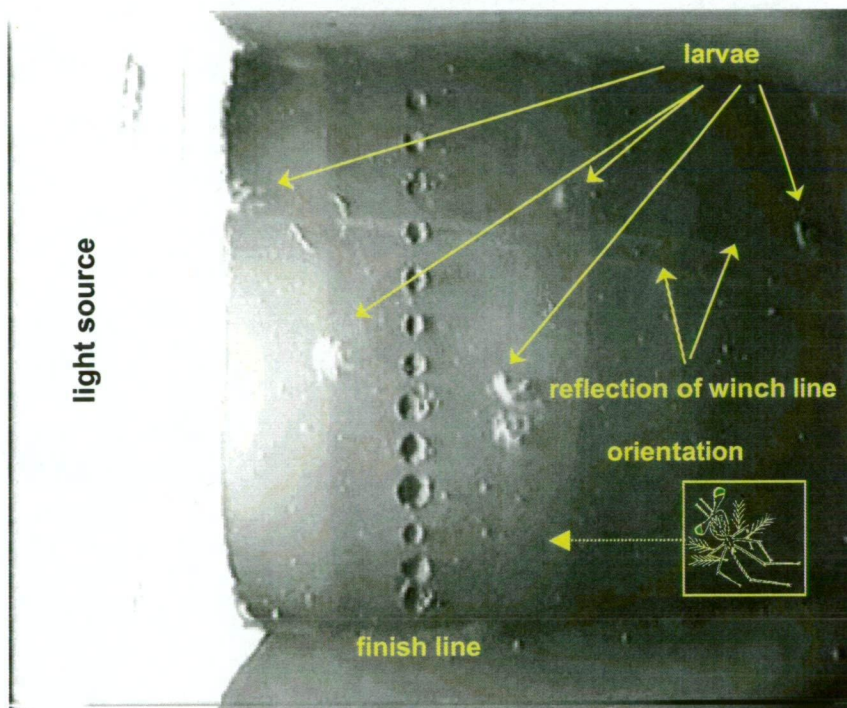
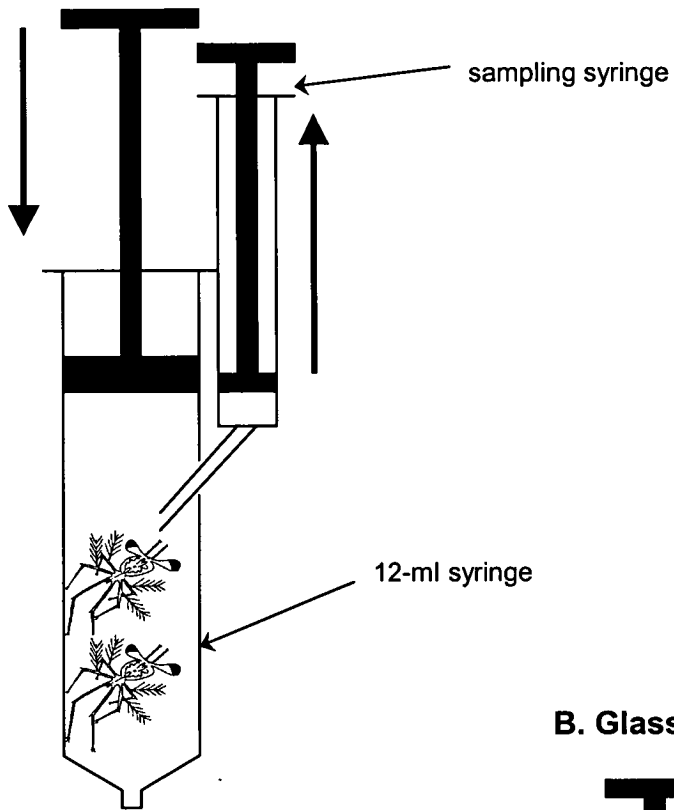


Figure 3.3 Frame view of newly-hatched *Jasus edwardsii* larvae swimming towards a light source. Swimming speed was measured from the point of release from the cage (Fig. 3.1.A) to the 'finish line' (distance of 10 cm). A notch on the winch line (reflecting at the surface of the water), which does not appear on this view, indicated the time of release from the cage.

A. Syringe respirometer



B. Glass respirometer

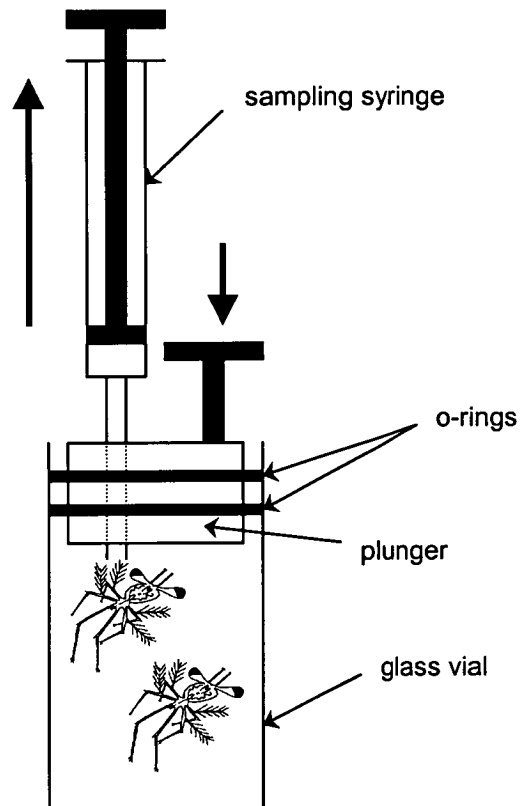


Figure 3.4 Schematic view of the syringe respirometer (A) and of the glass respirometer (B) used to measure the oxygen consumption of *Jasus edwardsii* larvae under different temperatures and light intensities, respectively.

3.4 Results

3.4.1 Behavioural and physiological responses to temperature

3.4.1.1 Geotaxis

Temperature had a significant effect on the behaviour of *J. edwardsii* larvae (PT, $F_{6,14}=14.54$, $P<0.0001$; Fig. 3.4). There was a significant decline in negative geotaxis from hatching to stage II (G-G univ., $F_{1,40, 19.55} = 74.86$, $P<0.0001$) which was accompanied by a shift in response through development (G-G univ., $F_{8,34, 19.55} = 3.25$, $P<0.05$). Indeed, in newly-hatched larvae, the loss of negative geotaxis observed under increasing temperature (ANOVA, $F_{6,14} = 9.84$, $P<0.001$) was significantly marked only above 16.0°C (LSD, $P<0.05$), while the decline in negative geotaxis seen at mid-stage I (ANOVA, $F_{6,14} = 4.06$, $P<0.05$) was significant (LSD, $P<0.05$) already at temperatures above 10.3°C (Fig. 3.4). There was also a significant effect of temperature on the geotactic response of stage II larvae (ANOVA, $F_{6,14} = 3.03$, $P<0.05$). This latest observation should, nonetheless, be interpreted cautiously due to the small number of larvae that were responsive to the experiment at this stage.

3.4.1.2 Physiology

The oxygen consumption of *J. edwardsii* larvae increased with increasing temperature, following a sigmoidal pattern (Table 3.3; Fig. 3.5) within the range of temperature tested at stage I ($R^2_{\text{adj}} = 0.741$, $P<0.0001$), stage II ($R^2_{\text{adj}} = 0.745$, $P<0.0001$) and stage III ($R^2_{\text{adj}} = 0.809$, $P<0.0001$). The Kimura Likelihood Ratio test indicated a significant ontogenic change in the respiratory response of phyllosomas over the three stages ($df = 4$, $\chi^2 = 53.02$, $P<0.0001$). There was no difference in the shape of the sigmoid response from stage I to stage III (Fig. 3.5 and Table 3.4) since development did not significantly affect either coefficient a ($df = 1$, $\chi^2 = 3.46$, $P = 0.063$), b ($df = 1$, $\chi^2 = 0.95$, $P = 0.330$), or x_0 ($df = 1$, $\chi^2 = 2.22$, $P = 0.136$). However, there was a significant ontogenic change in weight specific

$\dot{V}O_2$ ($df = 1$, $\chi^2 = 4.61$, $P < 0.05$) as indicated by the difference between models at the intercept level (y_0). According to the significant decline in the intercept of the logistic model from stage I to stage II ($df = 1$, $\chi^2 = 4.15$, $P < 0.05$), and from stage I to stage III ($df = 1$, $\chi^2 = 4.78$, $P < 0.05$), weight specific oxygen consumption decreased with development. The Q_{10} s (mean \pm SD, $n = 3$) grouped by stage for the temperature intervals of 9.9-14.0°C, 14.0-18.0°C and 18.0-21.9°C were 2.10 ± 0.88 , 2.73 ± 0.26 and 1.34 ± 0.19 , respectively. The Q_{10} fluctuated significantly within the range of temperature tested (Welch ANOVA, $F_{2,3.51} = 23.5$, $P < 0.01$) and was significantly higher in the 14-18°C range than between 18 and 22°C (G-H, $P < 0.01$).

Nitrogen excretion increased linearly with temperature at stage I ($R^2_{adj} = 0.47$, $F_{1,68} = 61.14$, $P < 0.0001$), stage II ($R^2_{adj} = 0.48$, $F_{1,103} = 95.25$, $P < 0.0001$) and stage III ($R^2_{adj} = 0.56$, $F_{1,38} = 50.35$, $P < 0.0001$) (Fig. 3.6 and Table 3.5). All three regression lines were parallel (ANCOVA, $F_{2,209} = 0.12$, $P = 0.889$) indicating a similar rate of increase in nitrogen excretion in stages I, II, and III larvae in relation to temperature. Weight specific nitrogen excretion levels dropped significantly with age (ANCOVA, $F_{2,211} = 122.15$, $P < 0.0001$) as stage I larvae excreted significantly more nitrogen per unit of weight than stage II (ANCOVA, $F_{1,172} = 194.49$, $P < 0.0001$) and III (ANCOVA, $F_{1,107} = 181.83$, $P < 0.0001$) larvae. However, there was no difference in excretion levels between stage II and III larvae (ANCOVA, $F_{1,142} = 2.15$, $P = 0.145$).

The feeding response to temperature was described at each stage with a quadratic model (Table 3.3) for the purpose of comparing the ontogenic change in the feeding rate increase with temperature (Fig. 3.7 and Table 3.6). Note that at stage III, however, the feed intake relationship (y in $\mu\text{g Artemia mg DW}^{-1} \text{ h}^{-1}$) with temperature (x in °C) is better described (Table 3.3) with the following linear regression ($R^2_{adj} = 0.75$, $F_{1,59} = 177.24$, $P < 0.0001$):

$$y = -2.96 + 0.41 x$$

There were marked developmental changes in the feeding response to temperature from stage I to stage III (KLR, $df = 3$, $\chi^2 = 321.18$, $P < 0.0001$). This

ontogenic difference in feeding is mainly attributed to a shift in the slope of the feed intake response to a temperature gradient (KLR, $df = 1$, $\chi^2 = 7.24$, $P < 0.01$). The decline of the regression slope with development, from stage I to stage III (KLR, $df = 1$, $\chi^2 = 6.80$, $P < 0.01$), and from stage II to stage III (KLR, $df = 1$, $\chi^2 = 4.54$, $P < 0.05$), showed a significant age related loss in temperature sensitivity in terms of feeding. To a lesser extent, ontogenic differences in feed intake were also observed at the higher end of the temperature range tested (KLR on c , $df = 1$, $\chi^2 = 3.87$, $P < 0.05$). There was indeed a tendency for the relative decline in feed intake observed in the first two stages to be absent at stage III. This was indicated by the KLR test on the coefficient c of the quadratic expression between stage I and III ($df = 1$, $\chi^2 = 3.24$, $P = 0.072$), and stage II and III ($df = 1$, $\chi^2 = 3.67$, $P = 0.055$). Although the KLR test highlighted a significant change through development in the intercept of the model ($df = 1$, $\chi^2 = 6.10$, $P < 0.05$), this observation could not be associated directly with an ontogenic change in weight specific feed intake since the shape of the response curve varied between stages. Nonetheless, there was an overall ontogenic shift in weight specific feed intake (K-W on feed intake grouped by stage, $df = 2$, $\chi^2 = 86.31$, $P < 0.0001$). Feed consumption declined with development and was higher at stage I than at stage II (G-H, $P < 0.0001$), and again higher at stage II than at stage III (G-H, $P < 0.001$).

The CRI appeared to be lower in stages II and III larvae than in stage I larvae (Table 3.7) and although CRI was consistent from 14 to 22°C, it was lower at 10°C throughout development.

The O:N ratio markedly increased from stage I to stage II (Table 3.8) and lowest values were consistently observed at 22°C throughout development.

Table 3.2 The Akaike's Information Criterion and (coefficient of determination adjusted for degrees of freedom) obtained for the modelling of oxygen consumption and feed intake against temperature in stages I, II, and III *Jasus edwardsii* larvae. Values underlined indicate the most parsimonious model (i.e. AIC) and/or the model with the least unexplained error (i.e. R^2_{adj}). Bold values indicate the model selected for further analysis.

Model*	Oxygen consumption			Feed intake		
	Stage I	Stage II	Stage III	Stage I	Stage II	Stage III
(1) $y = a + bx$	—	—	—	198.10 (0.69)	142.00 (0.53)	<u>9.71 (0.75)</u>
(2) $y = a + bx + cx^2$	—	—	—	<u>193.53 (0.74)</u>	<u>136.49 (0.56)</u>	<u>11.59 (0.75)</u>
(3) $y = a + bx + cx^2 + dx^3$	-309.46 (0.73)	-278.26 (0.74)	-188.90 (0.81)	—	—	—
(4) $y = \frac{a}{1 + \left(\frac{x}{x_0}\right)^b}$	-308.56 (0.71)	-279.37 (0.74)	-187.22 (0.78)	—	—	—
(5) $y = y_0 + \frac{a}{1 + \left(\frac{x}{x_0}\right)^b}$	<u>-314.32 (0.74)</u>	<u>-279.38 (0.75)</u>	<u>-192.99 (0.81)</u>	—	—	—

* (1) linear regression; (2) quadratic regression; (3) cubic regression; (4) logistic model with three parameters; (5) logistic model with four parameters.

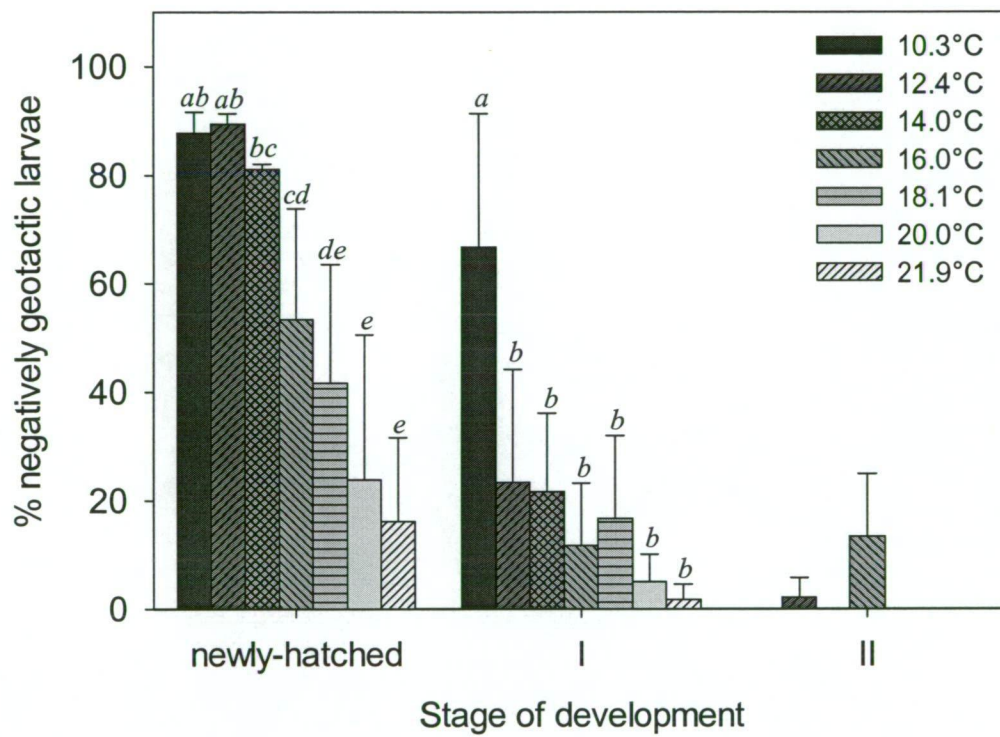


Figure 3.2 Mean (\pm SD) negative geotaxis in newly-hatched, stage I and II *Jasus edwardsii* larvae placed in the dark and exposed to temperatures ranging from 10.3°C to 21.9°C. Treatments with different letters differed significantly (LSD; $P < 0.05$).

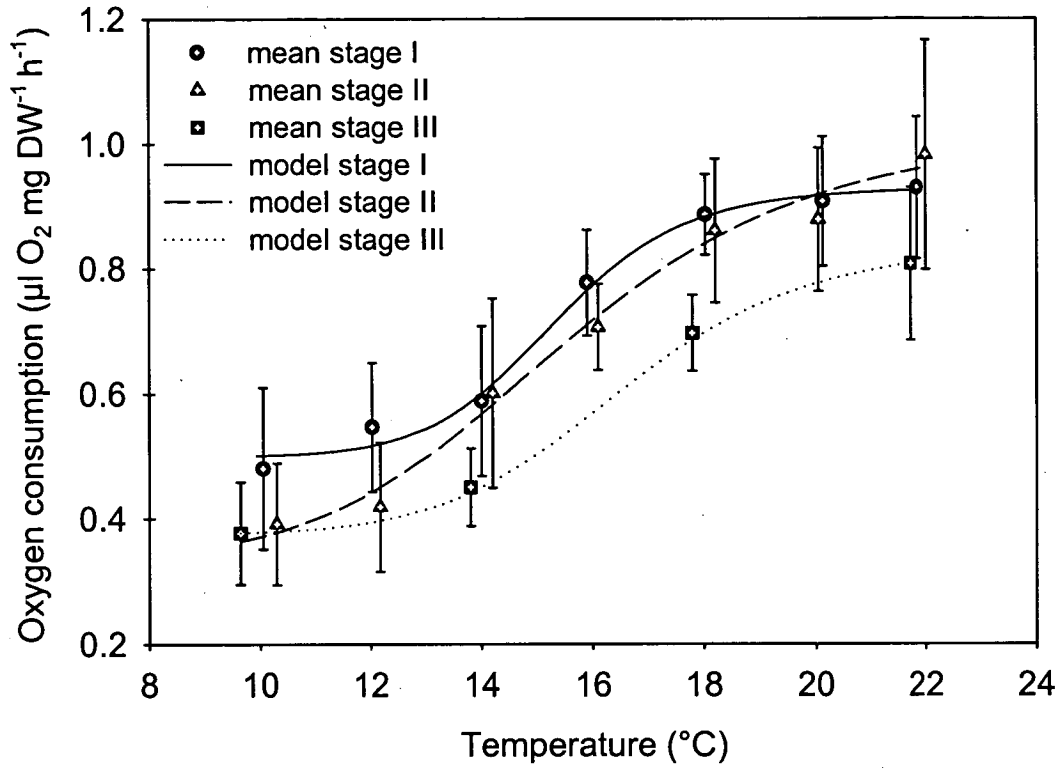


Figure 3.3 Mean (\pm SD) oxygen consumption of stage I, II and III *Jasus edwardsii* larvae exposed to different temperatures. A logistic expression was fitted to the raw data at each stage (Table 3.4).

Table 3.2 Parameters of the logistic expression fitted to the oxygen consumption of *Jasus edwardsii* larvae in response to temperature at stage I, II, and III (Fig. 3.5). Coefficients within a same row with different superscripts were significantly different (KLR, $P < 0.05$).

$$y = y_0 + \frac{a}{1 + \left(\frac{x}{x_0}\right)^b} *$$

Parameter	Stage I		Stage II		Stage III	
	Estimate	P	Estimate	P	Estimate	P
<i>a</i>	0.429	<0.0001	0.684	<0.001	0.463	<0.0001
<i>b</i>	-13.032	<0.001	-6.708	<0.05	-9.780	<0.01
<i>x</i> ₀	15.320	<0.0001	15.459	<0.0001	16.535	<0.0001
<i>y</i> ₀	0.500 ^a	<0.0001	0.336 ^b	<0.001	0.375 ^b	<0.0001

* where *y* is oxygen consumption (µl O₂ mg DW⁻¹ h⁻¹) and *x* is temperature (°C). This model was selected according to the procedure outlined in Table 3.3.

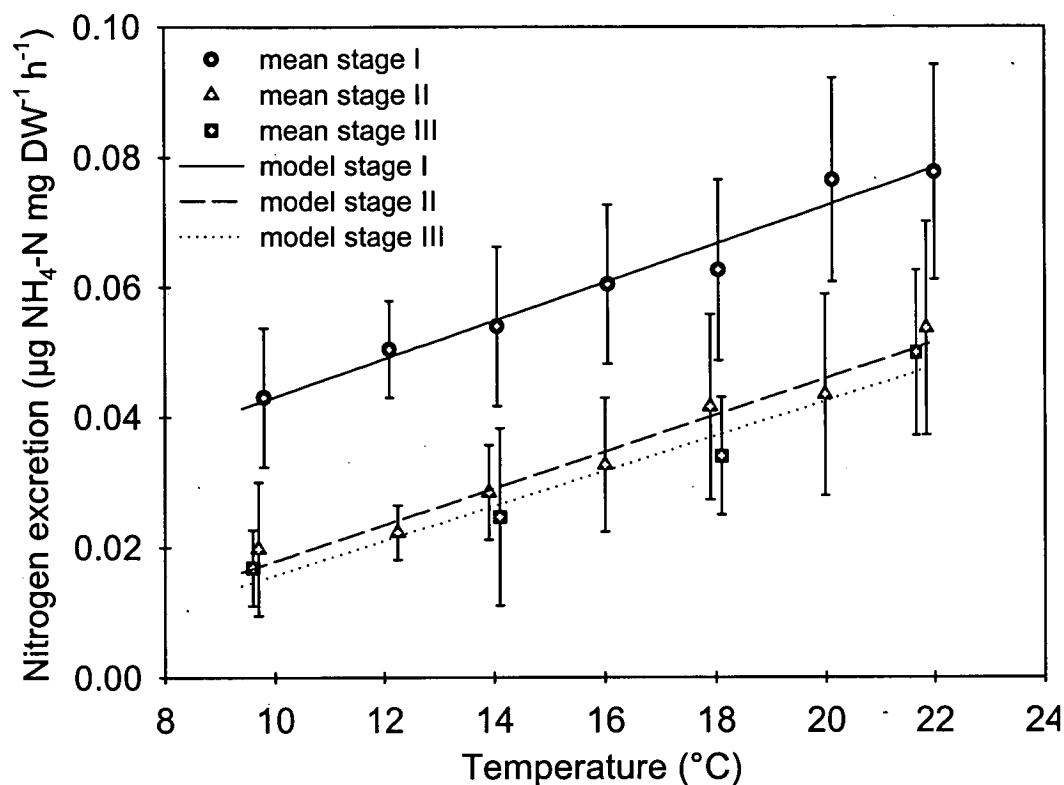


Figure 3.4 Mean (\pm SD) nitrogen excretion in stage I, II, and III *Jasus edwardsii* larvae exposed to different temperatures. A linear regression was fitted to the raw data at each stage (Table 3.5).

Table 3.2 Parameters of the linear regressions fitted to the nitrogen excretion of *Jasus edwardsii* larvae in response to temperature at stage I, II and III (Fig. 3.6). Coefficients with different superscripts were significantly different (ANCOVA, $P < 0.0001$).

$y = a + bx^*$						
	Stage I		Stage II		Stage III	
Parameter	Estimate	P	Estimate	P	Estimate	P
a	0.014 ^a	<0.05	-0.010 ^b	<0.05	-0.011 ^b	0.082
b	0.003	<0.0001	0.003	<0.0001	0.003	<0.0001

* where y is nitrogen excretion ($\mu\text{g NH}_4\text{-N mg DW}^{-1} \text{ h}^{-1}$) and x is temperature ($^{\circ}\text{C}$).

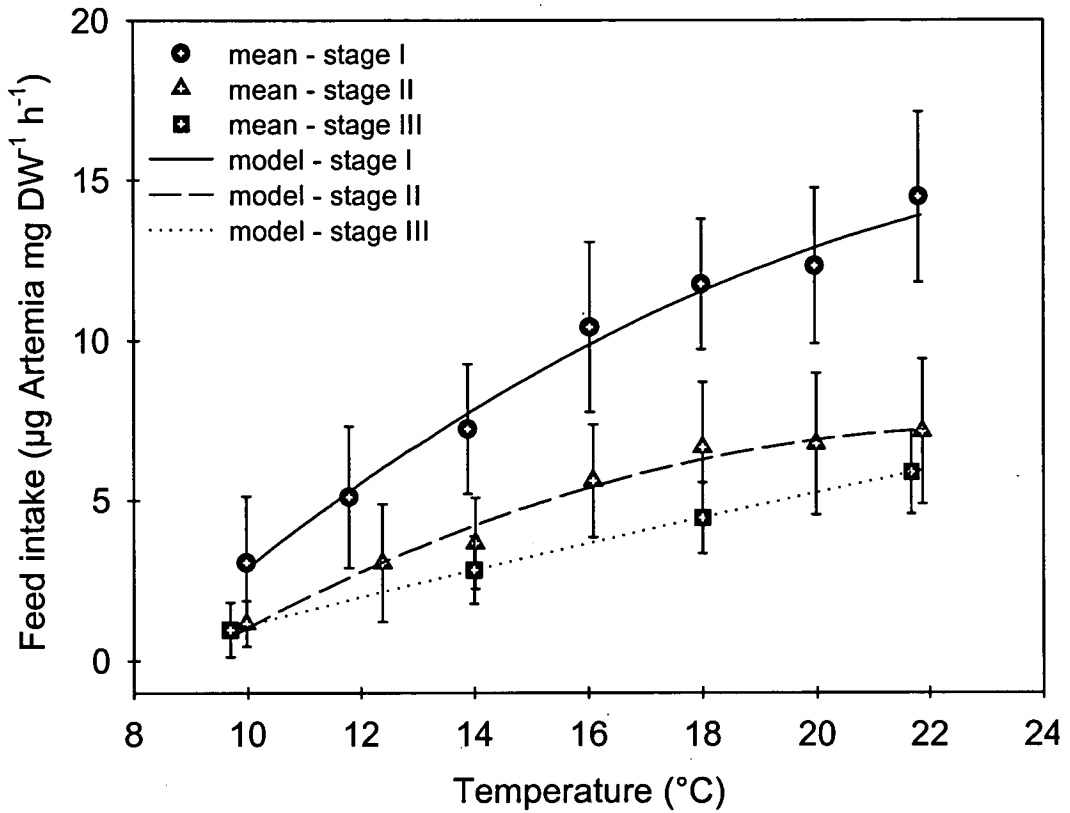


Figure 3.5 Mean (\pm SD) feed intake in stage I, II and III *Jasus edwardsii* larvae exposed to different temperatures. Raw data were fitted with a quadratic regression at each stage (Table 3.6).

Table 3.2 Parameters of the quadratic regressions fitted to the feed intake of *Jasus edwardsii* larvae in response to temperature at stage I, II and III (Fig. 3.7). Coefficients within a same row with a different superscript were significantly different (KLR, $P < 0.05$).

$$y = a + bx + cx^2 *$$

	Stage I		Stage II		Stage III	
Parameter	Estimate	P	Estimate	P	Estimate	P
<i>a</i>	-15.034	<0.001	-11.811	<0.0001	-3.622	0.079
<i>b</i>	2.188 ^a	<0.0001	1.633 ^a	<0.0001	0.502 ^b	0.073
<i>c</i>	-0.040 ^a	<0.05	-0.035 ^a	<0.01	-0.003 ^b	0.737

* where *y* is feed intake ($\mu\text{g Artemia mg DW}^{-1} \text{h}^{-1}$) and *x* is temperature ($^{\circ}\text{C}$). This model was selected according to the procedure outlined in Table 3.3.

Table 3.3 Convection requirement index (CRI) in stages I, II, and III *Jasus edwardsii* larvae exposed to different temperatures.

Larval stage	Temperature (°C)						
	10	12	14	16	18	20	22
I	11.0	16.1	21.1	22.5	22.4	22.7	26.0
II	3.0	7.3	6.1	7.9	7.7	7.7	7.3
III	2.6	—	6.3	—	6.4	—	7.3

Table 3.4 O:N ratios in stage I, II and III *Jasus edwardsii* larvae exposed to different temperatures.

Larval stage	Temperature (°C)						
	10	12	14	16	18	20	22
I	13.97	13.56	13.63	16.08	17.69	14.83	14.95
II	24.78	23.50	26.40	27.04	25.87	25.27	22.87
III	27.96	—	22.81	—	25.61	—	20.22

3.4.2 Behavioural and physiological response to light intensity

3.4.2.1 Behaviour

The swimming speed of newly-hatched larvae increased in a logarithmic fashion with increasing light intensity and is described by the following expression (linear regression, $r^2 = 0.356$, $F_{1,10} = 5.534$, $P < 0.05$; Fig. 3.8):

$$SS = -1.421 + 0.159(\log_{10}(LI))$$

where SS is the swimming speed (cm s^{-1}) of newly-hatched larvae and LI is the light intensity ($\text{quanta s}^{-1} \text{ cm}^{-2}$).

There was a significant difference in the response of phyllosoma exposed to conditions ranging from dark to high light intensity (PT, $F_{3,8} = 107.96$, $P < 0.0001$; Table 3.9). This pattern of response was consistent in newly-hatched, stage I, II, and III larvae (age, F stat., $F_{3,6} = 0.15$, $P = 0.924$), and did not significantly change across light conditions (i.e. dark, and low, medium and high light intensities) throughout early development (light \times age, F stat., $F_{9,24} = 0.964$, $P = 0.493$). For all age classes examined, exposure to light resulted in a significantly higher response under any of the light intensities tested than in the dark (Dunnett's comparison with control, $P < 0.01$, Table 3.9).

The phototactic response of phyllosoma changed significantly during the first 10 days of larval development (age, F stat., $F_{5,5} = 117.06$, $P < 0.0001$; Fig. 3.9). Furthermore, this shift in response occurred across all light intensities tested (age \times light, F stat., $F_{10,12} = 1.33$, $P = 0.317$). Newly-hatched larvae showed significant positive phototaxis under all light intensities tested (Dunnett's comparison with control, $P < 0.01$; Fig. 3.9). Although larvae became predominantly negatively phototactic from day 2 onwards (Fig. 3.9), positive phototaxis significantly increased (LSD, $P < 0.05$) late in stage I (i.e. day 6 and 8) and early in stage II (i.e. day 10) from the low response observed at day 4. Light intensity had a significant influence on the photoresponse of phyllosoma (PT, $F_{2,9} = 4.87$, $P < 0.05$) through the first 10 days of larval development. Although this effect was not detected within each age class it was particularly evident at day 2, 6 and 8 when the distribution of larvae exposed to low light intensity was not significantly different from the random distribution of animals in the dark control group (Fig. 3.9).

There was no significant change in photoresponse between mid-stages I, II and III (age, F stat., $F_{2,5} = 3.43$, $P = 0.115$), and no significant effect of light intensity within the range tested (light, PT, $F_{2,6} = 2.19$, $P = 0.194$; Fig. 3.10). In addition, the response to light intensity was constant with age (light x age, F stat., $F_{4,12} = 1.03$, $P = 0.430$). Nonetheless, the lack of significant difference between the distribution of responsive mid-stage I larvae subjected to low light intensity and in the dark (Dunnett's comparison with control, $P > 0.05$) contrasted with the predominantly negative phototaxis observed at mid-stages II and III under low light intensity.

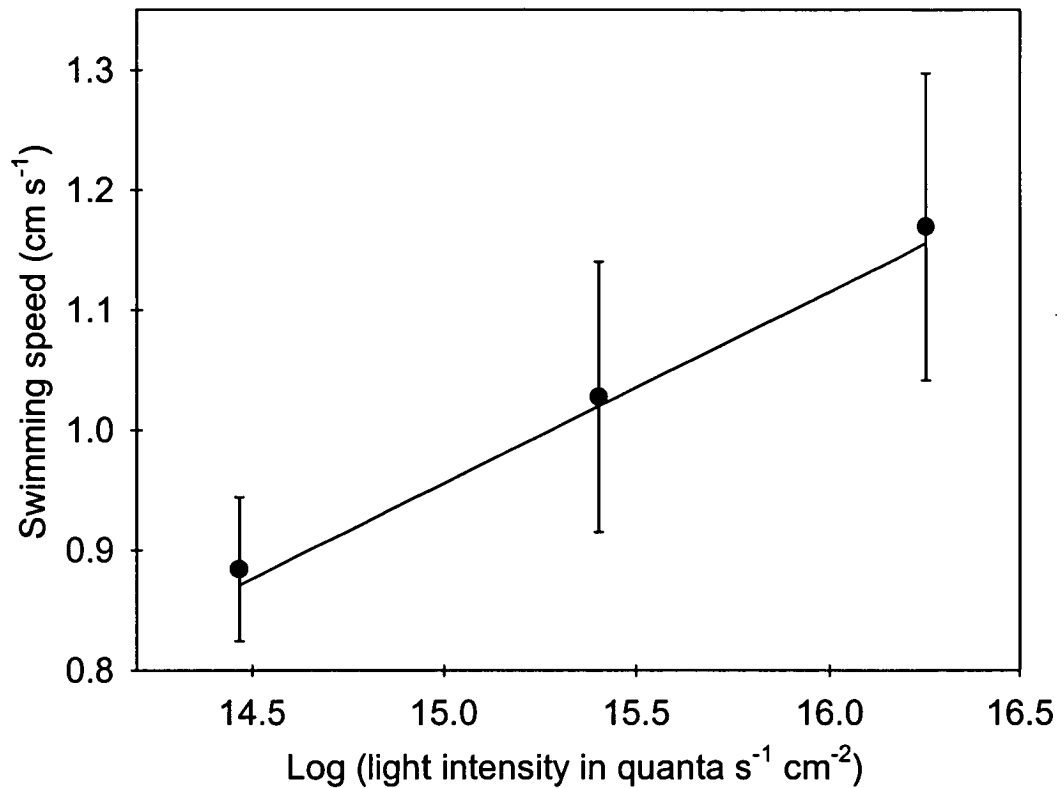


Figure 3.1 Mean (\pm SE) swimming speed of newly-hatched *Jasus edwardsii* larvae in relation to log-transformed light intensity. The relationship was fitted with a linear regression on the raw data.

Table 3.2 Mean (\pm SD) photoresponse (% larvae) observed in four age classes of early-stage *Jasus edwardsii* larvae under three light intensities and a dark control.

Stage	Light intensity (quanta s ⁻¹ cm ⁻²)			
	Dark control	Low 2.9 · 10 ¹⁴	Medium 2.5 · 10 ¹⁵	High 1.8 · 10 ¹⁶
Newly-hatched	57.50 ± 6.27	94.70 ± 4.33	94.80 ± 3.57	91.98 ± 8.71
Mid-stage I	61.35 ± 10.31	87.77 ± 6.88	94.38 ± 3.75	97.50 ± 5.00
Mid-stage II	60.56 ± 13.36	91.35 ± 4.22	96.71 ± 2.85	96.79 ± 2.78
Mid-stage III	46.11 ± 16.86	97.22 ± 2.55	97.78 ± 3.85	95.56 ± 5.09

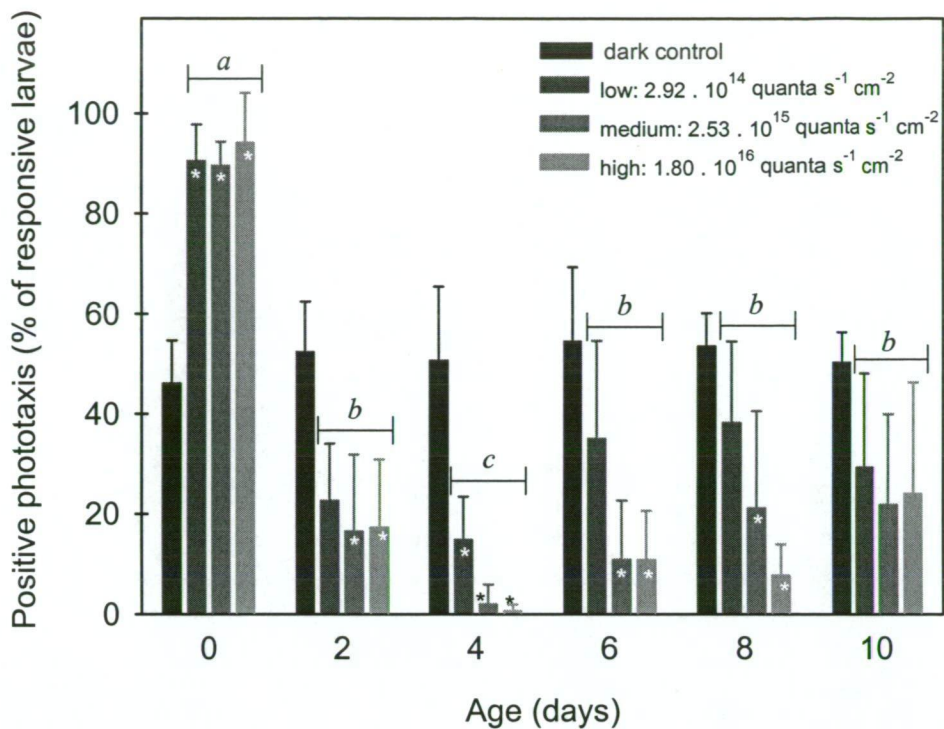


Figure 3.2 Mean (\pm SD) positive phototaxis in *Jasus edwardsii* larvae exposed to different light intensities from day 0 to day 10 (i.e. post-moult into stage II). Age classes with different letters differed significantly in phototaxis (LSD, $P < 0.05$). * significantly different from control within the same age class (Dunnett's test, $P < 0.05$).

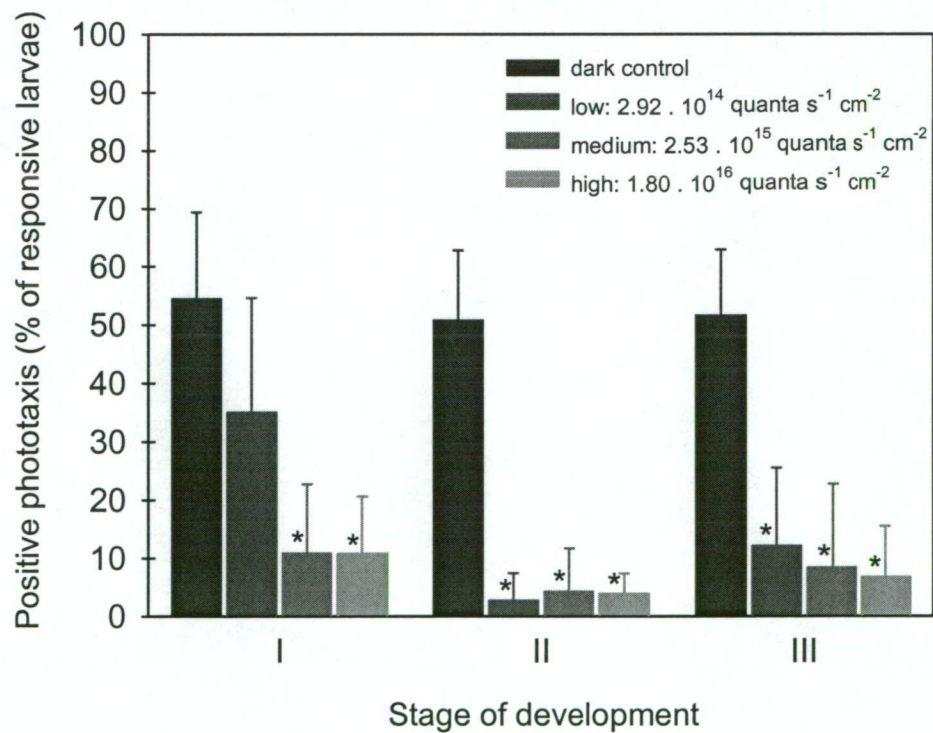


Figure 3.3 Mean (\pm SD) net photoresponse in *Jasus edwardsii* larvae exposed to different light intensities at mid-stages I (day 6), II (day 15-18) and III (day 25-30). * significantly different from control within the same age class (Dunnett's test; $P < 0.05$).

3.4.2.2 Physiology

The developmental decline in weight specific oxygen consumption was described with an exponential function (Table 3.10 and 3.11) for larvae in the dark ($R^2_{\text{adj}} = 0.59$, $F_{2,47} = 35.82$, $P < 0.0001$), larvae exposed to low light intensity ($R^2_{\text{adj}} = 0.85$, $F_{2,46} = 133.28$, $P < 0.0001$) and larvae exposed to high light intensity ($R^2_{\text{adj}} = 0.73$, $F_{2,47} = 68.02$, $P < 0.0001$) (Fig. 3.11). Light intensity had a significant effect on $\dot{V}O_2$ throughout development from hatching to stage V (KLR, $df = 3$, $\chi^2 = 118.99$, $P < 0.0001$). According to the difference in the coefficient b of the model (i.e. inflection of the exponential response), the shape of the oxygen consumption response through time was affected by light intensity (KLR, $df = 1$, $\chi^2 = 4.55$, $P < 0.05$). The initial ontogenic exponential decline in oxygen consumption was significantly larger in larvae exposed to low light intensity than in larvae in the dark (KLR on b , $df = 1$, $\chi^2 = 4.49$, $P < 0.05$). However, there was no effect of light intensity on the overall $\dot{V}O_2$ decline from hatching to stage V (KLR on a , $df = 1$, $\chi^2 = 2.72$, $P = 0.099$). The intercept of the model was also influenced by light intensity (KLR, $df = 1$, $\chi^2 = 12.39$, $P < 0.001$), and the comparison of intercepts between curves of significantly similar shape indicated that larvae exposed to high light intensity showed significantly higher $\dot{V}O_2$ than larvae in the dark throughout early development (Table 3.11).

Nitrogen excretion declined in a curvilinear fashion during development. Data were fitted with a rational expression (Table 3.10, Table 3.12 and Fig. 3.12) for the response observed in the dark ($R^2_{\text{adj}} = 0.43$, $F_{1,42} = 33.93$, $P < 0.0001$), under low light intensity ($R^2_{\text{adj}} = 0.49$, $F_{1,41} = 41.38$, $P < 0.0001$), and under high light intensity ($R^2_{\text{adj}} = 0.53$, $F_{1,43} = 50.28$, $P < 0.0001$). There was an overall effect of light intensity on the nitrogen excretion of phyllosoma throughout the developmental period examined (KLR, $df = 2$, $\chi^2 = 20.80$, $P < 0.0001$). The amplitude of the decline in nitrogen excretion through time (i.e. coefficient b in the rational expression) was not significantly different between the three light intensities tested (KLR, $df = 1$, $\chi^2 = 0.91$, $P = 0.340$). However, there was an overall effect of light intensity on the excretory rate of phyllosoma (KLR on a , $df = 1$, $\chi^2 = 15.37$, $P < 0.0001$), and nitrogen excretion was lower in the dark than in

low (KLR, $df = 1$, $\chi^2 = 8.19$, $P < 0.01$) and high (KLR, $df = 1$, $\chi^2 = 14.73$, $P < 0.001$) light intensity as indicated by ad-hoc testing between the intercept (a) at each light intensity (Table 3.12).

The weight specific consumption of *Artemia* declined in a curvilinear fashion throughout early development as described by the rational expression (Table 3.10, Table 3.13 and Fig. 3.13) for larvae in the dark ($R^2_{adj} = 0.69$, $F_{1,92} = 208.48$, $P < 0.0001$), larvae under low light intensity ($R^2_{adj} = 0.69$, $F_{1,92} = 204.87$, $P < 0.0001$), and larvae under high light intensity ($R^2_{adj} = 0.64$, $F_{1,92} = 166.13$, $P < 0.0001$). There was a significant effect of light intensity on feed intake throughout development (KLR, $df = 2$, $\chi^2 = 16.90$, $P < 0.001$). Although the shape of the response was consistent across treatments (KLR on b , $df = 1$, $\chi^2 = 1.12$, $P = 0.290$), light intensity significantly influenced the feed intake level of phyllosoma during early ontogeny (KLR on a , $df = 1$, $\chi^2 = 9.43$, $P < 0.01$). Feed intake by larvae in the dark was significantly lower than in larvae under low ($df = 1$, $\chi^2 = 8.69$, $P < 0.01$) and high light intensity ($df = 1$, $\chi^2 = 6.30$, $P < 0.05$), but there was no difference in *Artemia* consumption between larvae under low and high light intensity ($df = 1$, $\chi^2 = 0.05$, $P = 0.816$).

The CRI appeared to decline during early larval development and from mid-stage I to mid-stage V the CRI was consistently higher in larvae exposed to low light intensity than in larvae in the dark and under high light intensity (Table 3.14).

The O:N ratio increased with development and there was no clear pattern of response between the three light conditions tested (Table 3.15).

Table 3.2 The Akaike's Information Criterion and (coefficient of determination adjusted for degrees of freedom) obtained for the modelling of oxygen consumption, nitrogen excretion and feed intake against age in *Jasus edwardsii* larvae exposed to dark, low or high light intensity. Values underlined indicate the most parsimonious model (i.e. AIC) and/or the model with the least unexplained error (i.e. R^2_{adj}). Bold values indicate the model selected for further analysis.

Model*	Oxygen consumption			Nitrogen excretion			Feed intake		
	Dark	Low	High	Dark	Low	High	Dark	Low	High
(1) $y = a + bx + cx^2$	-195.2 (0.58)	-181.5 (0.78)	-172.3 (0.73)	-346.1 (0.42)	-324.4 (0.47)	-324.9 (0.53)	282.3 (0.63)	305.2 (0.65)	329.8 (0.57)
(2) $y = \frac{a}{1 + bx}$	<u>-195.7</u> (0.58)	-176.9 (0.75)	-167.6 (0.69)	<u>-348.4</u> (0.43)	<u>-329.8</u> (0.49)	<u>-325.6</u> (0.53)	<u>275.2</u> (0.65)	<u>299.6</u> (0.66)	<u>319.6</u> (0.61)
(3) $y = a \times e^{-bx}$	-193.4 (0.56)	-166.2 (0.69)	-161.2 (0.65)	-348.3 (0.43)	-325.9 (0.46)	<u>-327.0</u> (<u>0.54</u>)	283.1 (0.62)	309.4 (0.63)	329.9 (0.57)
(4) $y = y_0 + a \times e^{-bx}$	<u>-191.1</u> (0.59)	<u>-199.1</u> (0.85)	<u>-173.5</u> (0.73)	-346.7 (0.42)	-328.9 (<u>0.54</u>)	-325.1 (0.53)	301.1 (<u>0.66</u>)	334.5 (<u>0.67</u>)	341.6 (0.61)

* (1) quadratic regression; (2) rational expression with two parameters; (3) exponential function with two parameters; (4) exponential function with three parameters.

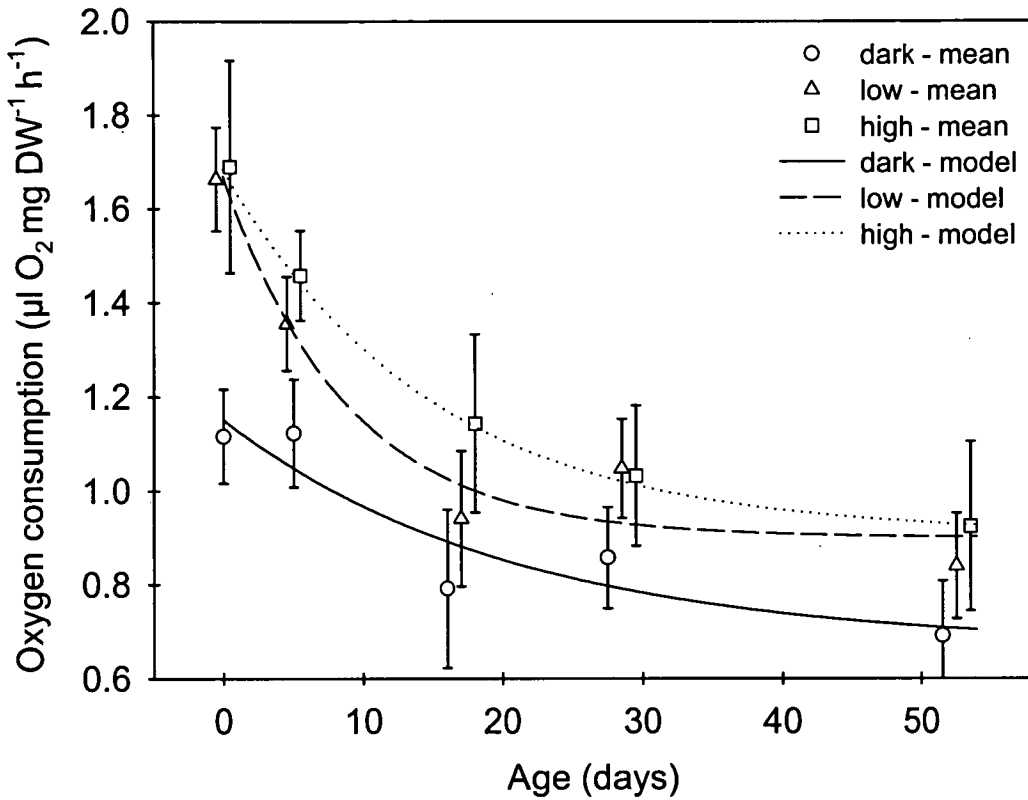


Figure 3.2 Mean (\pm SD) oxygen consumption in early stage *Jasus edwardsii* larvae in the dark, and under low ($7.7 \cdot 10^{12}$ quanta $s^{-1} cm^{-2}$) and high ($3.9 \cdot 10^{14}$ quanta $s^{-1} cm^{-2}$) light intensity. An exponential expression was fitted to the raw data at each light intensity (Table 3.11).

Table 3.2 Parameters of the exponential expression applied to the oxygen consumption in early stage *Jasus edwardsii* larvae exposed to different light intensities (Fig. 3.11). Coefficients within a same row with different superscripts were significantly different (KLR, $P < 0.05$).

$$y = y_0 + a \times e^{(-b \times x)} *$$

Parameter	Light intensity					
	Dark		Low		High	
	Estimate	P	Estimate	P	Estimate	P
y_0	0.668 ^a	<0.0001	0.900 ^b	<0.0001	0.907 ^b	<0.0001
a	0.484	<0.0001	0.770	<0.0001	0.777	<0.0001
b	0.048 ^a	<0.05	0.113 ^b	<0.0001	0.068 ^{a,b}	<0.001

* where y is oxygen consumption ($\mu l O_2 mg DW^{-1} h^{-1}$) and x is age (days). This model was selected according to the procedure outlined in Table 3.10.

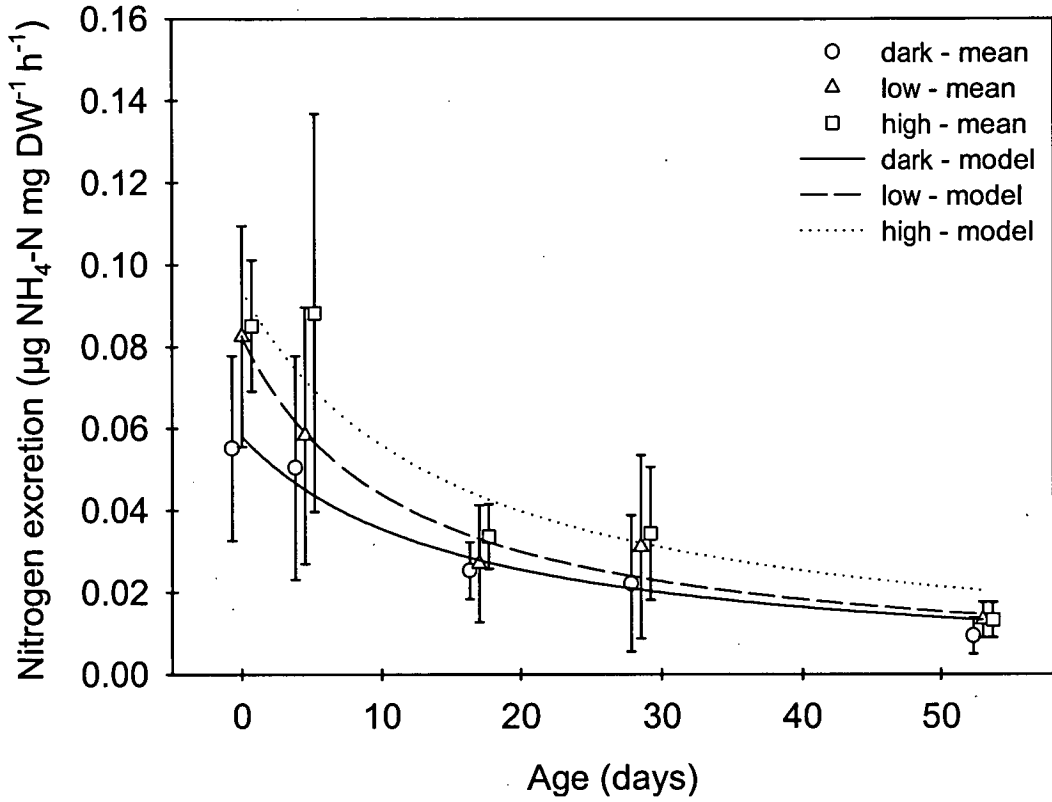


Figure 3.3 Mean (\pm SD) nitrogen excretion in early stage *Jasus edwardsii* larvae in the dark, under low ($7.7 \cdot 10^{12}$ quanta $s^{-1} cm^{-2}$) and high ($3.9 \cdot 10^{14}$ quanta $s^{-1} cm^{-2}$) light intensity. A rational expression was fitted to the raw data at each light intensity (Table 3.12).

Table 3.2 Parameters of the rational expression applied to the nitrogen excretion in early stage *Jasus edwardsii* larvae exposed to different light intensities (Fig. 3.12). Coefficients within a same row with different superscripts were significantly different (KLR, $P < 0.05$).

$$y = \frac{a}{1 + bx} *$$

Parameter	Light intensity					
	Dark		Low		High	
	Estimate	P	Estimate	P	Estimate	P
<i>a</i>	0.058 ^a	<0.0001	0.083 ^b	<0.0001	0.094 ^b	<0.0001
<i>b</i>	0.064	<0.01	0.088	<0.01	0.068	<0.01

* where *y* is nitrogen excretion ($\mu g NH_4-N mg DW^{-1} h^{-1}$) and *x* is age (days). This model was selected according to the procedure outlined in Table 3.10.

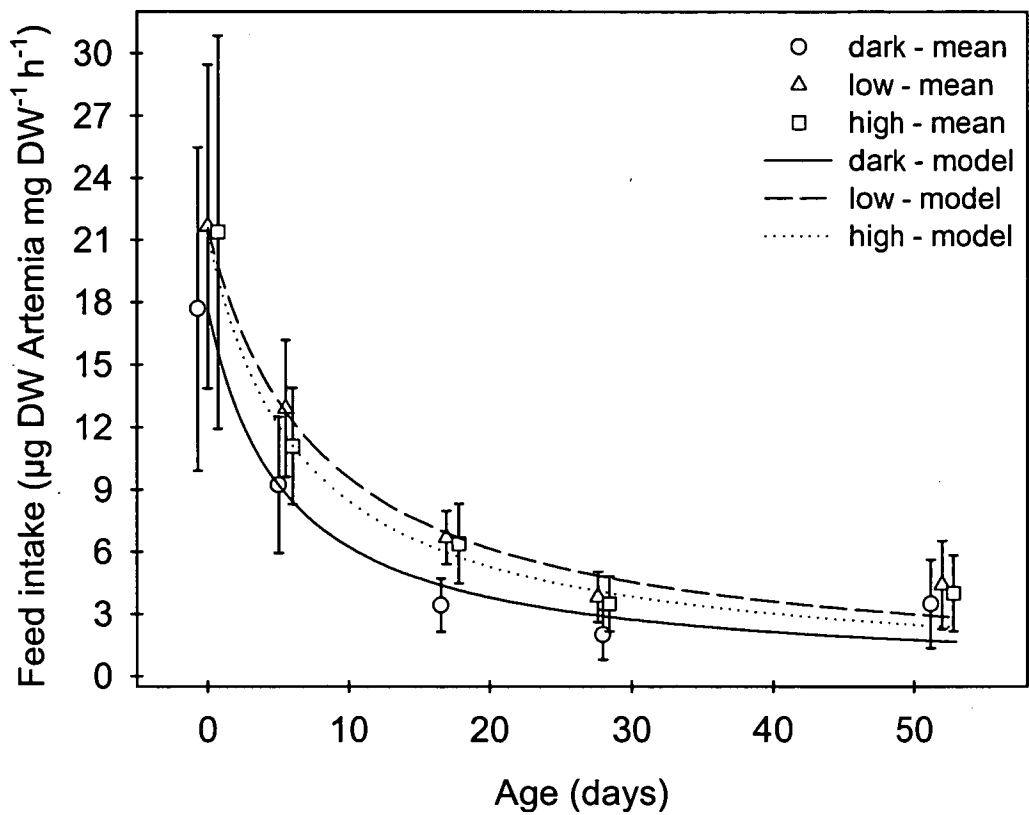


Figure 3.4 Mean (\pm SD) feed intake in early stage *Jasus edwardsii* larvae in the dark, under low ($7.7 \cdot 10^{12}$ quanta $s^{-1} cm^{-2}$) and high ($3.9 \cdot 10^{14}$ quanta $s^{-1} cm^{-2}$) light intensity. A rational expression was fitted to the raw data at each light intensity (Table 3.13).

Table 3.2 Parameters of the rational expression applied to the feed intake in early stage *Jasus edwardsii* larvae exposed to different light intensities (Fig. 3.13). Coefficients within a same row with different superscripts were significantly different (KLR, $P < 0.05$).

$$y = \frac{a}{1 + bx} *$$

Parameter	Light intensity					
	Dark		Low		High	
	Estimate	P	Estimate	P	Estimate	P
a	17.666 ^a	<0.0001	21.579 ^b	<0.0001	21.261 ^b	<0.0001
b	0.183	<0.0001	0.126	<0.0001	0.152	<0.0001

* where y is feed intake (μg Artemia $mg DW^{-1} h^{-1}$) and x is age (days). This model was selected according to the procedure outlined in Table 3.10.

Table 3.3 Convection requirement index (CRI) computed for newly-hatched, and stages I, II, III and V *Jasus edwardsii* larvae subjected to different light intensities.

Larval stage	Light intensity		
	Dark	Low	High
Newly-hatched	15.85	13.02	12.65
I	8.21	9.51	7.61
II	4.32	7.11	5.59
III	2.33	3.64	3.37
V	5.03	5.24	4.33

Table 3.4 O:N ratios in newly-hatched, and stages I, II, III and V *Jasus edwardsii* larvae exposed to different light intensities.

Larval stage	Light intensity		
	Dark	Low	High
Newly-hatched	25.30	25.20	24.82
I	27.83	29.05	20.65
II	39.08	43.53	42.46
III	48.41	42.15	37.54
V	92.38	79.15	87.13

3.5 Discussion

3.5.1 Behaviour

Light and gravity are the principal orienting stimuli governing the directional response of animals in the vertical plane (Fraenkel and Gunn, 1961). However, the signs of response and their amplitude can be altered by other environmental parameters such as light intensity, temperature and salinity (Sulkin, 1984). While gravity is essentially a constant parameter with depth, light is highly variable in the marine environment. Factors such as season, time of the day, depth, water quality and weather conditions can all affect light intensity and spectral composition (Clarke, 1970). In decapod larvae, light can act as a stimulus in both the locomotor activity of the animal and its orientation (see review by Sulkin, 1984). In this study on *J. edwardsii* phyllosoma larvae light stimulated both locomotor activity and phototaxis. The swimming speed of phyllosomas increased logarithmically with increasing light intensity thereby tending toward a maximum at the highest intensity tested ($1.8 \cdot 10^{16}$ quanta $\text{s}^{-1} \text{cm}^{-2}$). Mikami (1995) reported a similar logarithmic pattern in the swimming speed of newly-hatched *Thenus orientalis* larvae exposed to illuminance of different intensities. The effect of light intensity on swimming speed has been reported in other larval decapod species (topic reviewed in Sulkin, 1984). The presence of photokinesis in *J. edwardsii* larvae implies their ability to vary locomotor activity according to ambient lighting and this may be reflected in their metabolic rate.

The change in body orientation or phototaxis caused by light has been studied in many decapod larvae for its evident link with the diel vertical migration behaviour of zooplankton. Diel modes of depth regulation are generally classified as nocturnal or reverse migration patterns (see review by Haney, 1988). In the nocturnal migration pattern, zooplankters swim upward towards the end of the day and spend the night in the upper waters. This behaviour contrasts with the reverse migration pattern in which the animals move downward around dusk and spend the night at depth. Newly-hatched *J. edwardsii* larvae were positively phototactic

under all light intensities tested in the present study. Positive phototaxis was also reported in the newly-hatched phyllosoma of *Panulirus cygnus* (Ritz, 1972) and *Thenus orientalis* (Mikami, 1995). In *P. cygnus* however, Ritz (1972) found an upper intensity threshold of 4.6 mW cm^{-2} at which the positive response disappeared. By contrast, newly-hatched *J. edwardsii* larvae remained attracted to a light source of higher intensity ($1.8 \cdot 10^{16} \text{ quanta s}^{-1} \text{ cm}^{-2}$ or 6.0 mW cm^{-2} ; see light intensity unit conversion in Appendix V). This may suggest dissimilarity between the early dispersal strategy of *J. edwardsii* and *P. cygnus* larvae since depth regulation, governed in part by light intensity, plays an important role in advective transport (Phillips and McWilliam, 1986).

From positively phototactic at day 0, *J. edwardsii* larvae reversed their response by day 2 and from this stage in their development larvae appeared to be predominantly negatively phototactic. However, during stage I, negative phototaxis appeared as a gradual response to increasing light intensity. Furthermore, in 2, 6 and 8 days old stage I larvae, the population sampled under low light intensity was evenly distributed between positive and negative photosensitive animals. This implies that the lowest light intensity tested in the present work ($2.9 \cdot 10^{14} \text{ quanta s}^{-1} \text{ cm}^{-2}$) approached the illuminance threshold of phototaxis reversal in mid-stage I *J. edwardsii* larvae. This level of light is below the range of illuminance of 50 to $250 \mu\text{E m}^{-2} \text{ s}^{-1}$ ($3.0 \cdot 10^{15}$ to $1.5 \cdot 10^{16} \text{ quanta s}^{-1} \text{ cm}^{-2}$), which limits the vertical distribution of early stage *P. cygnus* larvae within depth of 30 to 60 m in the middle of the day (Rimmer and Phillips, 1979). This suggests that *J. edwardsii* larvae would reach greater depth than their western Australian counterparts. Because of the many factors that can modify the light threshold in decapod larvae (see review by Sulkin, 1984), results obtained in the laboratory and in the field should nonetheless be compared cautiously.

The observations made on the photoresponse of phyllosoma throughout stage I indicate that *J. edwardsii* larvae would rapidly adopt a nocturnal diel migration pattern. Descending at depth during day light from day 2 after hatching (i.e. at 18°C), phyllosomas would not only be well adapted to an oceanic environment early on in their development but would also require a habitat where they could reach significant depth. Therefore one could speculate that larvae

hatched in large bays and estuaries could become trapped by the hydrodynamics of these coastal features and consequently become lost to recruitment. Although this concept remains hypothetical, it could be linked to the offshore migration of ovigerous *J. edwardsii* females towards the deep seaward part of reefs reported in New Zealand (McKoy and Leachman, 1982; MacDiarmid, 1991). McKoy and Leachman (1981) suggested that offshore movement of females in areas of strong water currents would assure larval dispersal away from the coast where larvae would escape the high predatory pressure of the reef studied by Kingsford and MacDiarmid (1988). Furthermore, developing into nocturnal diel vertical migrators would provide larvae with an additional strategy against the loss of pre-recruits to early predation (Haney, 1988).

Older larvae at mid-stage II and III showed consistent movement away from the light from $2.9 \cdot 10^{14}$ to $1.8 \cdot 10^{16}$ quanta $s^{-1} cm^{-2}$. By contrast with stage I animals there was no sign of a possible reversal in phototaxis at the lowest intensity tested, suggesting a decline in light threshold with age. This could indicate the descent of larvae older than stage I to lower strata as they move offshore, similar to the ontogenic change in depth distribution found by Rimmer and Phillips (1979) in *P. cygnus* larvae. However, factors other than light intensity such as hydrostatic pressure (Ennis, 1975; Schembri, 1982; Gardner, 1996), temperature (Forward, 1990) and the presence of thermoclines (McConnaughey and Sulkin, 1984; Boudreau *et al.*, 1991; Gardner, 1998) can influence depth regulation in zooplankton and should be investigated in *J. edwardsii* larvae.

The hypothesis of a nocturnal migration pattern in early-stage *J. edwardsii* larvae drawn here from laboratory experiments is in agreement with data obtained in field surveys (Booth, 1994; Bruce *et al.*, 2000). Booth (1994), for instance, found that late stage larvae became more common in the upper 100 m 1-2 h after sunset than during the afternoon. Bruce *et al.* (2000) indicated that *J. edwardsii* larvae of indeterminate stages occurred at the surface in broad daylight, before 0800 h and after 1300 h. These observations contrast with the predominant negatively phototactic behaviour reported earlier in this chapter. Starvation was found to increase the occurrence of positive phototaxis in *Rhithropanopeus*

harrisii larvae (Cronin and Forward, 1980) and may influence the photoresponse of *J. edwardsii* larvae in the wild, driving them into nutrient rich surface waters.

Temperature has been reported to play an important role in the depth regulation of decapod larvae since it affects both their body orientation and the locomotor activity that modifies their buoyancy (Sulkin, 1984). The geotactic response of decapod larvae to temperature is generally characterised by upward swimming at low temperatures and active downward swimming or passive sinking at elevated temperatures (Ott and Forward, 1976; McConnaughey and Sulkin, 1984; Forward, 1990; Boudreau *et al.*, 1991; Gardner, 1998). Newly-hatched *J. edwardsii* larvae were predominantly negatively geotactic from 10 to 14°C, and the distribution of the animals gradually shifted downward from 16 to 22°C. Although, the possible effect of temperature on the rate of activity of phyllosoma was not tested in the present study, the trend observed in newly-hatched larvae is comparable to the downward shift of *Rhithropanopeus harrisii* and *Callinectes sapidus* stage I larvae exposed to extreme warm temperatures that, Ott and Forward (1976) and McConnaughey and Sulkin (1984) respectively attributed to a cessation of locomotor activity. The negative response to gravity in newly-hatched *J. edwardsii* larvae between 10 and 14°C implies that despite the absence of light phyllosoma would remain at or near the surface within this range of temperatures. These thermal conditions are within the range of sea surface temperatures recorded from southern to northern Tasmanian waters (i.e. 8.6 to 17.4°C⁽²⁾) during the period of larval release in the wild (Winstanley, 1977).

The gradual decline in negative geotaxis with increasing temperature that was observed in newly-hatched larvae was absent at mid-stage I. At this stage, gravity in the absence of light, was the predominant cue for depth regulation in phyllosomas at temperatures ranging from 12 to 22°C. Below this range however, the larvae swam to the surface. This observation suggests that the sensing of cold water (i.e. ~10°C) during vertical migration would stimulate swimming and/or a shift in the body orientation of *J. edwardsii* phyllosoma. Such behaviour is similar to the avoidance of cold water reported in *R. harrisii* and *Neopanope sayi* (Forward, 1990), and in *Homarus americanus* (Boudreau *et al.*, 1991). Avoidance

⁽²⁾ NOAA satellite AVHRR imagery.

of cold temperature was no longer observed in mid-stage II larvae within the thermal range tested. Therefore at this stage, larvae could tolerate colder temperatures to possibly reach greater depth. Alternatively, temperature might cease to be a factor for depth regulation in stage II larvae. The prevalence of *J. edwardsii* phyllosomas at the surface at night, at temperatures ranging predominantly from 12.2 to 15.0°C (Bruce *et al.*, 2000) would suggest that to maintain their buoyancy by way of locomotor activity under these conditions, stage II and possibly older larvae would have to respond to one or a combination of the following factors: (1) the increase in hydrostatic pressure with depth (Kelly *et al.* 1982; Schembri, 1982); (2) low light intensity levels originating from the moon or the stars (Rimmer and Phillips, 1979); (3) circadian triggers such as dusk, dawn (Sulkin *et al.*, 1979) and/or changes in angular light distribution (Schalleck, 1942; 1943).

3.5.2 *Physiology*

In the present study, oxygen consumption, nitrogen excretion and feed intake were measured as indicators of the physiological response of early-stage *J. edwardsii* larvae placed under different temperature and light intensity regimes. The rates of oxygen consumption reported here should be regarded as routine metabolism in unrestrained animals (Ikeda *et al.*, 2000). There was, however, a marked discrepancy between the results obtained at 18°C in the temperature experiment and those reported under similar ambient conditions in the light intensity trial in which, oxygen consumption rates were consistently 9 and up to 64% higher than in the temperature trial. Although the methods used in both trials were similar in many aspects (Table 3.2), differences in extrinsic factors (e.g. lighting conditions, design of the respirometer) and/or intrinsic factors (e.g. larval nutritional status) may have hypothetically caused marked variations in the measurement of oxygen uptake. The direct overhead fluorescent light used in the temperature trial contrasted with the more diffuse lighting provided in the light intensity trial. A possible difference in larval photobehaviour between these two conditions (Schalleck, 1942; 1943) could have a noticeable effect on locomotor

activity and therefore on metabolism. Furthermore, the elongated and narrow syringes used in the temperature trial might have promoted greater confinement of the larvae than the glass respirometers designed with a wider base. Consequently the level of activity might have been lower in the syringe than in the glass respirometer. Oikawa and Itazawa (1995) reported on a decline in oxygen consumption with increased confinement in *Cyprinus carpio*. Additionally, the larval nutritional status might have been different between the two trials. Indeed, in the temperature trial, larvae were adapted for 24 h to their experimental temperature during which period they were fed in a static vessel. In these conditions, the specific dynamic action (post-prandial metabolic pulse) linked with the level of food ingested (Kiørboe *et al.*, 1985; Chapter 3 in this thesis) could have been lower than in larvae sampled directly from the rearing tanks (i.e. light intensity trial), where turbulent mixing is likely to increase the rate of encounter with preys and therefore feed intake (Dower *et al.*, 1997). The above reasoning remains speculative and further research is required to clarify this issue which is of considerable importance in future work on the comparative physiology of *J. edwardsii* larvae. Nonetheless, the $\dot{V}O_2$ measurements obtained in this study are in close agreement with respiratory rates reported in phyllosoma of the spiny lobster *Panulirus interruptus* (Belman and Childress, 1973).

The general trend throughout this work was the decline of all three physiological parameters examined from stage I to stage II, which subsequently remained stable until stage V. Such change in weight specific response is common in decapod larvae and was previously reported with observations of oxygen consumption (Mootz and Epifanio, 1974; Agard, 1999) and nitrogen excretion (Capuzzo and Lancaster, 1979; Agard, 1999). Of these two metabolic parameters, most reports are on the ontogenic decline in $\dot{V}O_2$. According to Prosser (1973), an ontogenic decline in oxygen consumption is due to the disproportionate increase in tissue of low metabolic rate as the organism grows and develops. In *Palaemon serratus* larvae, the decline in weight specific oxygen consumption from stage IV to stage V was explained by a sudden increase in the number of cells in the hepatopancreas, and by an increase in enzymatic activity due to the acclimation of the organism to its new diet (Yagi *et al.*, 1990).

Similarly, Agard (1999) suggested that the developmental decline in metabolic rate observed in *Macrobrachium rosenbergii* larvae was due to morphological changes in the hepatopancreas. In *J. edwardsii* larvae the development of the hepatopancreas, which is clearly visible during the first stage (personal observation) together with acclimation to a new diet might also explain the early ontogenic decline in oxygen consumption. A developmental decline in weight specific oxygen consumption is nonetheless not a general trend in crustacean larvae as Capuzzo and Lancaster (1979) reported on an increase in $\dot{V}O_2$ with development in *Homarus americanus*, and Johns (1981) found no change in $\dot{V}O_2$ throughout the larvae development of *Cancer irroratus*.

Although several authors have found a relative decline and/or a depression in oxygen consumption at elevated acclimation temperatures (Halcrow and Boyd, 1967; Schatzlein and Costlow, 1978; Newell and Branch, 1980), the sigmoid pattern observed in *J. edwardsii* larvae at stage I, II and III has seldom been reported in other decapod larvae. Such response could primarily be attributed to the range of temperature tested in relation to the specific thermal preferendum of *J. edwardsii* larvae. The Q_{10} analysis for the range of temperatures tested matched the sigmoid pattern of oxygen consumption particularly as the significant decline of the Q_{10} from the 14-18°C range (i.e. 2.73) to the 18-22°C range (i.e. 1.34) corresponded with the flattening of the $\dot{V}O_2$ response at high temperatures. This suggests a decline in the rate of $\dot{V}O_2$ increase at high temperature (i.e. 18-22°C) similar to observations made by Halcrow and Boyd (1967) in *Gammarus oceanicus*, and by Schatzlein and Costlow (1978) in the larvae of *Emerita talpoida* and *Libinia emarginata*. The behavioural response of *J. edwardsii* to temperature could explain the inflection of the curve at cold temperatures in stage I larvae since under these conditions phyllosoma were reported to swim actively upwards. However, the behavioural response to temperature in stage II animals provides no support for a sigmoid pattern of oxygen consumption at this stage. It should be stressed that the different behavioural, biochemical and physiological reactions that make up the overall metabolism of an animal are known to respond to temperature at different rates (Willmer *et al.*, 2000). It is therefore possible that extreme high temperatures would cause some metabolic processes to slow their

activity while under conditions of extreme low temperature an organism would be unable to reduce the rate of some physiological processes below a vital level. If this were the case in *J. edwardsii* larvae, the extreme temperature tested here would be beyond the thermal preferendum for the species. However, before this can be confirmed the capacity of *J. edwardsii* larvae to adjust their metabolism through sufficient acclimation (i.e. leading to acclimatization (Clarke, 1987)) must be assessed.

The weight specific nitrogen excretion of *J. edwardsii* larvae followed a linear increase with rising temperature at a similar rate in all three stages examined. This implies that a greater amount of protein is being metabolised following a temperature rise. Since in zooplankton, nitrogen excretion is linked to food availability (Corner and Cowey, 1968; Appendix VI), and because experimental animals were fed prior to measurements of excretion, the level of nitrogen output observed in the present study is likely to have been influenced by the feeding rate of phyllosoma during acclimation at different temperatures. However, in stage I and II larvae, feeding activity showed a relative decline in its response rate at high temperature, in agreement with reports by Tong *et al.* (2000) working on the same species. The implications of these contrasting observations between nitrogen excretion and feed intake are two fold. Firstly, at high temperatures, nitrogen excretion is only partly linked to the nutritional status of phyllosoma. Therefore, the lack of incline in the nitrogen excretion curve at the warm end of the thermal range tested could be a sign of physiological stress under sub-optimal conditions. And secondly, the potential of phyllosoma to compensate for increased energy losses through protein utilisation at high temperatures is diminished. Consequently, growth would be reduced in *J. edwardsii* larvae reared under conditions approaching temperatures at the warmer end of the experimental range tested in the current experiment. These findings are in agreement with results obtained in Chapter 2 in which reduced growth and survival were reported at 21.5°C. However, the present suggestion of reduced growth potential at high temperature contrasts with the evidence of sustained growth and increased development reported by Tong *et al.* (2000) for early stage *J. edwardsii* larvae reared from 18 to 24°C. Nonetheless, Tong *et al.* (2000) rightfully acknowledged

that their observations, which came in spite of a relative reduction in food consumption at high temperature, may have been influenced by past nutritional history. These authors suggested that at high temperatures their experimental animals previously mass reared under optimal conditions (i.e. as we know them so far) might have drawn on reserves in excess of the 'point of reserve saturation' (Anger and Dawirs, 1981) to move to the next stage.

Although, changes in the rate of response to temperature may be indicators of stress (Schatzlein and Costlow, 1978), the measurement of individual physiological functions does not necessarily provide evidence that an organism's potential for survival or growth will be reduced (Brett, 1958). The convection requirement index (CRI) and the O:N ratio provide a better assessment of environmental stress with respect to the nutritional status and the growth potential of the animal (Bayne, 1973; Newell and Branch, 1980; Johns, 1981). The convection requirement index was used in the present study to compare the potential energy gain or loss by phyllosoma at different temperatures and light intensities. Reflecting the respiratory and feeding responses of *J. edwardsii* larvae to temperature, the CRI was relatively constant between 14 and 22°C in all three stages examined. This suggests that energy intake and energy losses are balanced within this temperature range.

The O:N ratio is commonly used in zooplankton to provide information on the nature of the substrate oxidised (Corner and Cowey, 1968; Ikeda *et al.*, 2000). Carbohydrates make up 5% at most of the dry weight of zooplankton (Raymont and Conover, 1961), which is not enough to support an animal's metabolic requirement for 24 h (Ikeda *et al.*, 2000). Therefore, zooplankton must rely essentially on protein and lipid as their main metabolic substrates. In ammonotelic animals, the ratio of oxygen respired and nitrogen excreted is approximately 8 by atoms when metabolism is chiefly protein oriented (Ikeda, 1974). Furthermore, in zooplankton, an O:N ratio higher than 8 indicates an increased reliance on lipid as metabolic substrate, while an O:N ratio greater than 24 corresponds to a lipid-dominated catabolism (Ikeda, 1974; Mayzaud and Conover, 1988). Consequently, in *J. edwardsii* larvae, the marked increase in

O:N ratios from stage I to stage II indicated an increase in lipid catabolism with development. Such a shift in metabolic substrate could coincide with the development of the digestive system discussed earlier. However, this observation contrasts with reports of ontogenic decline in O:N ratio in *Homarus americanus* (Sasaki *et al.*, 1986) and in *Hyas araneus* (Anger, 1991) larvae. As highlighted by Mayzaud and Conover (1988), the value of the O:N ratio depends essentially on the nutritional situation faced by the animal. Indeed, if feed intake is less than the metabolic requirements, the animal considered may increase its reliance on lipid as energy substrate in order to spare amino-acids from the oxidative process (Huggins and Munday, 1968). If this was the case in *J. edwardsii* larvae, the developmental increase in O:N ratio observed in the present study would reflect the inappropriateness of the diet used in culture rather than an ontogenic change in nutritional requirements. The O:N ratio of *J. edwardsii* larvae placed at 22°C remained consistently below values obtained at lower temperatures during the first three stages of development. Indeed, a low O:N ratio at high temperature resulted from a relative decline in oxygen consumption accompanied by a linear increase in nitrogen excretion. Therefore, the increased protein catabolism for maintenance at high temperature represents a net loss to growth. Johns (1981) and Agard (1999) reported on the effect of temperature on the O:N ratio in larvae of *C. irroratus* and *M. rosenbergii* respectively. These authors also found a tendency for larvae to shift toward a protein-oriented metabolism under environmental conditions (i.e. temperature and salinity) determined as sub-optimal for growth.

Depending on the ambient temperature, locomotor activity can represent between 52 and 82% of the metabolic demand of active newly-hatched *J. edwardsii* larvae (Chapter 4). Therefore, any external factor such as light that can influence locomotor activity in decapod larvae (Sulkin, 1984) may have significant repercussions on an animal's metabolism. In *J. edwardsii* larvae, all three physiological functions examined (i.e. oxygen consumption, nitrogen excretion and feed intake) were influenced by light. The increased $\dot{V}O_2$ observed in larvae subjected to light (i.e. low and high light intensities) when compared to

larvae in the dark, prevailed throughout early development to stage V. Overall, the response by larvae exposed to low and high light intensities did not differ in terms of oxygen consumption, nitrogen excretion and feed intake. These results suggests that the main effect of light intensity in phyllosoma is found between total darkness and light, and that under light phyllosoma would perform equally at low or high light intensity, at least for the range of light intensities tested in the present study. Since light at the levels experienced by phyllosoma in the present study is unlikely to have a direct influence on metabolism, the increase in VO_2 in larvae exposed to light may be attributed primarily to increased locomotor activity. Indeed, the effect of light on locomotor activity and responsiveness of larvae was clearly demonstrated earlier in this chapter. In agreement with the present findings, Kils (1979) reported a 30% increase in oxygen consumption in *Euphausia superba* under light than in animals in darkness. Nonetheless, the effect of illuminance on metabolism is not a widespread phenomenon in zooplankton and Pearcy *et al.* (1969) did not find any difference in oxygen consumption between *Euphausia pacifica* placed in the dark and subjected to light.

Weight specific nitrogen excretion levels were elevated under light, and as with the increased VO_2 , coincided with increasing locomotor activity. This suggests that *J. edwardsii* larvae use protein as a metabolic substrate for propulsion. This observation has significant implications in the design of a rearing system since excessive locomotor activity could potentially impair growth. Although, metabolic energy losses increase under light, they may be compensated by the greater feed intake seen in larvae subjected to low and high light intensities as opposed to larvae in the dark. These results are consistent with findings by Moss *et al.* (1999) working on the same species, and with reports of higher feeding rates under light than in the dark in *Rhithropanopeus harrissii* (Cronin and Forward, 1980) and *Ranina ranina* (Minagawa, 1994) larvae. From mid-stage I and in subsequent stages, *J. edwardsii* larvae placed under low light intensity showed consistently higher CRI than larvae in the dark, which is partly explained by the difference in feeding rates between the two treatments. Although these results were not directly statistically validated, they tend to

indicate that larvae exposed to low light intensity have a greater metabolic feeding efficiency than phyllosomas placed in the dark. A similar difference in CRI was observed between low and high light intensity but given the lack of statistically significant differences in the oxygen uptake and feeding rate between the two light intensities, a similar interpretation would be speculative.

The relatively high values of O:N ratio obtained in this study are the consequence of the higher oxygen uptake recorded in the light intensity trial than in the temperature experiment. As in the temperature experiment, the O:N ratio increased from newly-hatched to stage V indicating that in culture conditions, *J. edwardsii* larvae tend to rely more on lipid as a metabolic substrate with development. As discussed earlier, it is not clear whether this shift in metabolic substrate is related to ontogenic changes in dietary requirements, or to a deterioration of the nutritional status in older larvae. There was, however, no clear shift in O:N associated with light intensity.

During daylight surface sampling at sea, Bruce *et al.* (2000) observed the highest densities of *J. edwardsii* larvae before 0800 h and after 1600 h. According to the results of the present study, the light levels experienced at these times of the day by phyllosoma may enhance their feeding rate. However, further work is required with a broader illuminance range to determine metabolic and feeding light thresholds and to investigate diurnal changes in feeding activity in *J. edwardsii* larvae.

3.6 Conclusions

The observations made on the behaviour of *J. edwardsii* larvae suggested that temperature affects geotaxis in stage I larvae but has no effect at stage II. By contrast the photoresponse of phyllosoma prevails throughout the early stages of development and is characterised by positive phototaxis at hatch, but this is reversed to negative phototaxis within 2 days. From an aquaculture perspective, the control of animal behaviour is essential in order to prevent the animals from being in prolonged contact with the walls of the rearing vessel, to avoid uneven larval distribution, and to raise the encounter rate with preys. The results obtained in the present study clearly indicated that light could be used to control the behaviour of stage I phyllosomas in the laboratory by adjusting the light intensity to a level approaching the threshold for reversal of phototaxis. A clearer understanding of the mechanisms underlying depth regulation in phyllosoma larvae is essential not only to further our understanding of larval migration and recruitment to the fishery but also to improve the design of culture systems.

The physiological data obtained throughout the present study provided valuable information on the metabolic processes underlying the effect of temperature and light intensity on the development and growth of *J. edwardsii* larvae. The energetic imbalance (i.e. reduced feed intake versus raised nitrogen excretion) at high temperatures (i.e. above 18°C) may cause depression in growth in early-stage *J. edwardsii*. Therefore, high temperatures should be avoided in culture until the capacity of phyllosoma to acclimatise to such conditions is assessed.

In view of the physiological results obtained with light intensity, a light phase is recommended for the culture of phyllosoma in order to maximise feed intake. This, followed by a dark phase of decreased larval metabolic activity may improve food conversion. During the light phase, intensities equal to or below $3.9 \cdot 10^{14} \text{ quanta s}^{-1} \text{ cm}^{-2}$ should be suitable for larval rearing. While higher light

intensities are likely to be inappropriate in culture given the nocturnal migration pattern of *J. edwardsii* larvae observed in the wild (Booth, 1994; Bruce *et al.*, 2000), lower light levels require further investigation considering the trends observed in the present study in terms of oxygen consumption, nitrogen excretion, feeding rate and behaviour.

3.7 References

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Chapter 4

The use of chemical
immobilisation to estimate
standard metabolism and the
energetic cost of swimming
in newly-hatched larvae

4.1 Abstract

The standard metabolic rate (SMR) of newly-hatched *Jasus edwardsii* larvae was obtained by measuring oxygen consumption in phyllosomas immobilised with 2-phenoxyethanol. The cost of swimming was calculated as the difference between oxygen consumption in active larvae (i.e. routine metabolic rate) and the standard metabolic rate of immobilised phyllosomas. Oxygen consumption increased with increasing temperature in both immobilised and swimming larvae. Oxygen consumption was higher in swimming larvae than in immobilised larvae at 10 and 15°C and not significantly different at 20°C. This implied that the swimming activity of larvae at 10 and 15°C allowed for maintenance of buoyancy or upward swimming as opposed to passive sinking at 20°C where swimming was minimal. These conclusions were validated from video examination of behavioural response. Negative geotaxis was significantly higher in larvae at 10 and 15°C (93.3 ± 2.9 and 85 ± 5 % \pm SD, respectively) than at 20°C (35 ± 8.7 % \pm SD). In addition, the level of activity was not significantly different between larvae at 10 and 15°C which showed similar rates of ascent (0.43 ± 0.24 and 0.42 ± 0.23 cm s⁻¹ \pm SD respectively) at these temperatures. The present study demonstrated that temperature is an important cue for depth regulation in *J. edwardsii* larvae. In addition, chemical immobilisation with 2-phenoxyethanol may be an expedient technique for the direct measurement of SMR in *J. edwardsii* larvae, and the respirometry method presented here was effective in studying the behavioural response of newly-hatched larvae.

4.2 Introduction

The energetics of swimming in aquatic animals was described by several authors as the increase in oxygen consumption associated with increasing activity (Foulds and Roff, 1976; Mickel and Childress, 1978; Cowles and Childress, 1988; Buskey, 1998). Experimentally, the cost of swimming is obtained from the difference between routine (i.e. oxygen consumption in unrestrained organisms) and standard metabolism (i.e. metabolism in a resting animal). Due to the technical difficulties in measuring standard metabolism alone, only a few studies have quantified the cost of locomotor activity in planktonic crustaceans. Estimations of the standard metabolic rate (SMR) can be obtained either through direct measurement on resting animals (Klyashtorin and Kuz'micheva, 1976), or extrapolated at zero activity from the modelling of swimming activity in response to a gradual stimulus (Halcrow and Boyd, 1967; Torres and Childress, 1983). Other workers have calculated SMR as the difference between routine metabolic rate (RMR) and cost of swimming, the later being estimated from the calculation of dead drag (Vlymen, 1970; Klyashtorin and Yarzhombek, 1973). Little information is available on anaesthesia as a means of immobilising aquatic animals for measuring standard metabolic rate. This method was briefly mentioned by Raymont (1983) and was previously used on herring (*Clupea harengus*) eggs (Holliday *et al.*, 1964), and on skate (*Raja erinacea*) (Hove and Moss, 1997) to measure resting metabolic rate and standard metabolism, respectively.

This study aimed at assessing the use of anaesthesia as a method for direct measurement of standard metabolic rate in order to define the energetic cost of swimming in *Jasus edwardsii* larvae. The energy expenditure for swimming was compared to behavioural response at different temperatures. Results were examined with respect to the applicability of anaesthesia in estimating standard metabolism and the cost of swimming in spiny lobster larvae.

4.3 Material and methods

4.3.1 Larvae

Larvae were obtained during the spring of 1998 and 1999 from berried females caught off the east coast of Tasmania, and brought to the TAFI Marine Research Laboratories, Hobart. Until hatching of the larvae, females were held at ambient temperature and fed pilchards. At hatching, temperature ranged from 14.9 to 16.5°C. Unless specified, trials were conducted with larvae from a single brood. To avoid interference from possible circadian behavioural patterns, the following trials were all conducted between 1000 and 1700 h.

4.3.2 *Preliminary studies: Oxygen consumption by resting, starved, and fed larvae*

The immobilisation of larvae for up to 6 h (period required to measure oxygen consumption in a closed respirometer) was achieved by bathing in a solution 2-phenoxyethanol (M.W. = 138.17; 1.109 g ml⁻¹) in seawater (1 ml l⁻¹). The steadiness of the metabolic rate of stage I larvae (n = 12, 6 from each of 2 different broods) in this resting state was measured through microscopic examination of heart pulse (Fig. 4.1). From its normal level in seawater, the heart rate of stage I larvae dropped significantly (P<0.0001; Kruskal-Wallis test on ranks; Fig. 4.2) in 2-phenoxyethanol and remained subsequently stable for 6 h (P>0.05; Dunn's method for multiple comparison). Treatment with 2-phenoxyethanol did not cause any mortality during the resting phase, and up to 48 h post-exposure. A starvation period of 17.5 h was determined to be sufficient to measure the oxygen consumption in post-absorptive stage I larvae (Appendix VI).

Following these preliminary findings, oxygen consumption ($\dot{V}O_2$) was measured in mid-stage I larvae under three different physiological states: (1) resting post-absorptive (RPA), (2) active post-absorptive (APA), and (3) active absorptive (AA). The larvae used in this experiment had been previously reared in six culture vessels (about 100 per 500 ml vessel) for four days, during which time they were fed *Artemia* nauplii and maintained at 17°C. Food was withheld

18 h prior to the start of the experiment in four of the six culture vessels (see Appendix VI). Starved larvae were used to measure $\dot{V}O_2$ in RPA and APA larvae, while $\dot{V}O_2$ in AA was assessed on larvae fed until their transfer into respirometers. For each physiological state, oxygen consumption was measured in five closed 12-ml syringe respirometers (see description in Chapter 3, Fig. 3.3) stocked with ten active larvae (five from two different vessels). The respirometers were filled with 0.2 μm filtered 34 ppt seawater with 25 ppm of oxytetracycline (Engemycin 100, Intervet, Australia) to minimise background microbial respiration. Two-phenoxyethanol was added to seawater in the RPA treatment. Respirometers were incubated in a waterbath at 17°C and larvae were acclimated to the syringe for at least 1 h before measuring oxygen consumption over a 4-h period following the procedure outlined in Chapter 3. Oxygen partial pressure was determined in 1-ml water samples using a BMS Mk2 (Radiometer, Copenhagen) connected to a Cameron Instrument Company BGM 300 blood gas analyser (Port Aransas, Texas). The electrode was calibrated to zero using a solution of sodium metabisulphite and to 100% saturation with aerated seawater. Oxygen consumption was expressed as $\mu\text{l O}_2 \text{ larva}^{-1} \text{ h}^{-1}$ after deduction of background respiration obtained from three control respirometers at each treatment. Data failed to meet the ANOVA assumption of homoscedasticity and the effect of physiological state on oxygen consumption was tested with the Kruskal-Wallis one way ANOVA on ranks (K-W). Differences between treatments were isolated with the Newman-Keuls method of multiple comparison (N-K). Analyses were carried out with Sigmastat 1.0 statistical software.

4.3.3 *Experiment 1: The effect of temperature on the metabolic rate of resting and active larvae*

Oxygen consumption was measured in newly-hatched larvae in an active and in a resting state, and at three different temperatures: 9.8, 15.0, and 20.0°C. $\dot{V}O_2$ measurements were carried out following the methods described above except that 10 larvae per respirometer were incubated for 6 h (including 1 h of acclimation) instead of 5 h, to allow for detection of oxygen consumption at the lowest temperature tested (i.e. 9.8°C). The equipment used to measure oxygen

saturation in 1-ml samples consisted of a polarographic electrode connected to a digital controller (Rank Brothers Ltd., UK). The lowest final level of dissolved oxygen observed ($4.77 \text{ ml O}_2 \text{ l}^{-1}$) was above the P_{crit} determined in stage I larvae (Chapter 5). The effect of physiological state and temperature on the oxygen consumption of newly-hatched larvae was analysed with a two way analyses of variance (2-ANOVA) after log transformation of the data in order to meet assumption of homoscedasticity. SPSS 10.0 statistical software was used for this analysis. The 'cost of swimming' ($\dot{V}\text{O}_2 \text{ active} - \dot{V}\text{O}_2 \text{ resting}$) was calculated at each temperature as an indicator of activity. The van't Hoffs rule's Q_{10} (Clarke, 1987) was computed for the resting and active metabolic rates, and for the cost of swimming following the formula given in Chapter 3.

4.3.4 Experiment 2: The effect of temperature on geotaxis

Following the methods described in Chapter 3, the geotactic response of newly-hatched larvae to temperature was tested in glass columns (320 x 100 x 100 mm) placed in aquaria maintained at 10.0, 15.0, and 20.1°C. There were three columns at each temperature and 30 larvae were introduced into each column. The effect of temperature on negative geotaxis of newly-hatched larvae was tested with ANOVA and the analysis was carried out using SPSS 10.0.

4.3.5 Experiment 3: The effect of temperature on the rate of ascent

The rate of ascent of newly-hatched larvae was examined at 9.9, 14.9 and 20.0°C. At each temperature larvae ($n \approx 100$) were placed in a glass column and left to acclimate in the dark for 1 h. After acclimation the positively phototactic newly-hatched phyllosomas (see Chapter 3) were attracted to the bottom of the column with a light source (Fig. 4.3). The upward swimming of larvae was recorded with a camera connected to a video recorder (Fig. 4.4). An infrared light placed above the column was used to visualise the animals in the dark. Video recordings were later analysed to determine the larval rate of ascent over a 10-cm vertical section in larvae ($n = 80$) not touching the column's walls. Mean rates of ascent at different temperatures were compared with a t-test using SPSS 10.0 statistical software.



Figure 4.1 Magnified ventral view of a stage I *Jasus edwardsii* phyllosoma. The heart is located in the circled area.

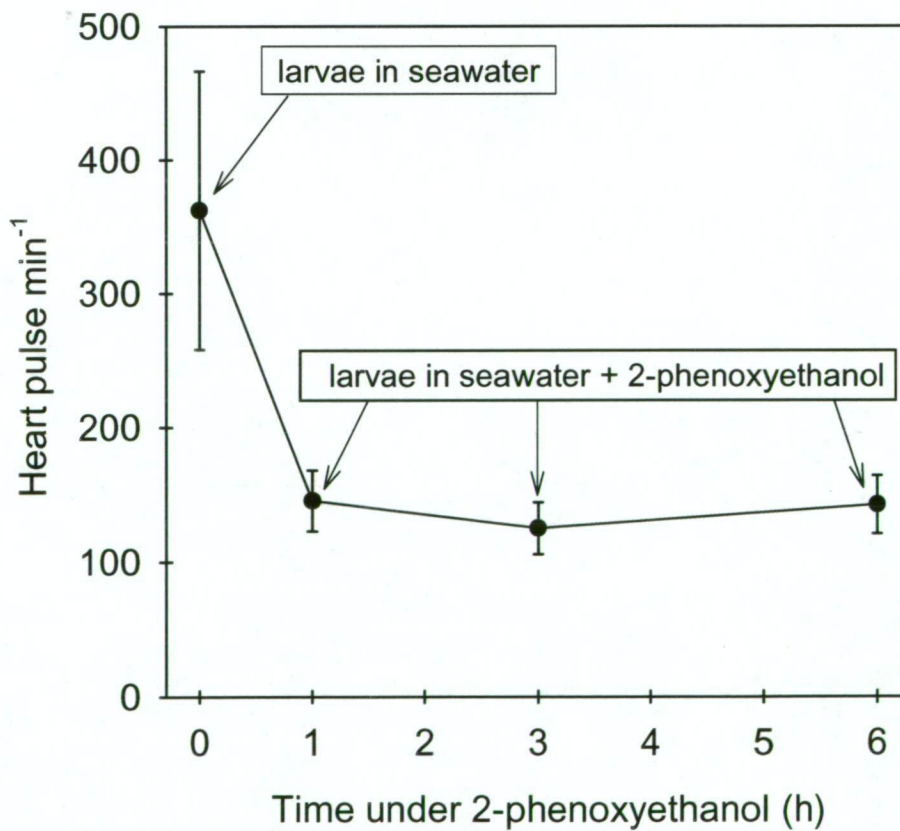


Figure 4.2 Mean (\pm SD) heart pulse rate (pulse min⁻¹) of stage I *Jasus edwardsii* larvae in a prolonged resting state under 2-phenoxyethanol.

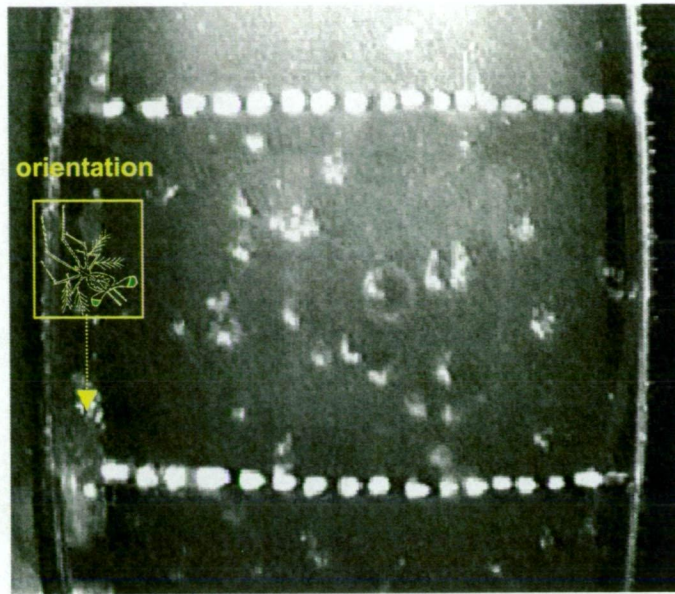


Figure 4.3 Frame view of newly-hatched *Jasus edwardsii* larvae in a water column, swimming downwards towards a light source.

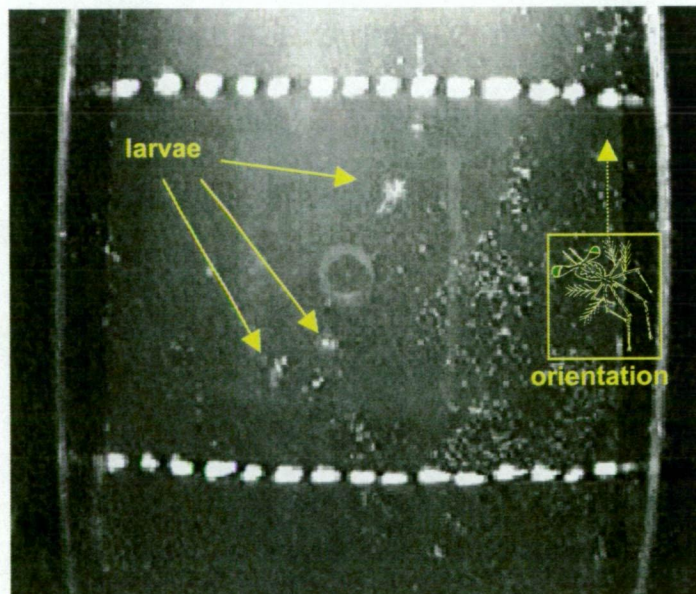


Figure 4.4 Frame view of newly-hatched *Jasus edwardsii* larvae migrating upwards in a water column. The rate of ascent was measured between the two dotted white lines.

4.4 Results

4.4.1 Preliminary experiment: Oxygen consumption by resting, starved, and fed larvae

Oxygen consumption of stage I larvae increased with increasing physiological demand (K-W, $df = 2$, $H = 11.4$, $P < 0.01$; Fig. 4.5). Oxygen consumption in RPA larvae was lower than in APA larvae, which was in turn less than oxygen consumption in AA larvae (N-K, $P < 0.05$).

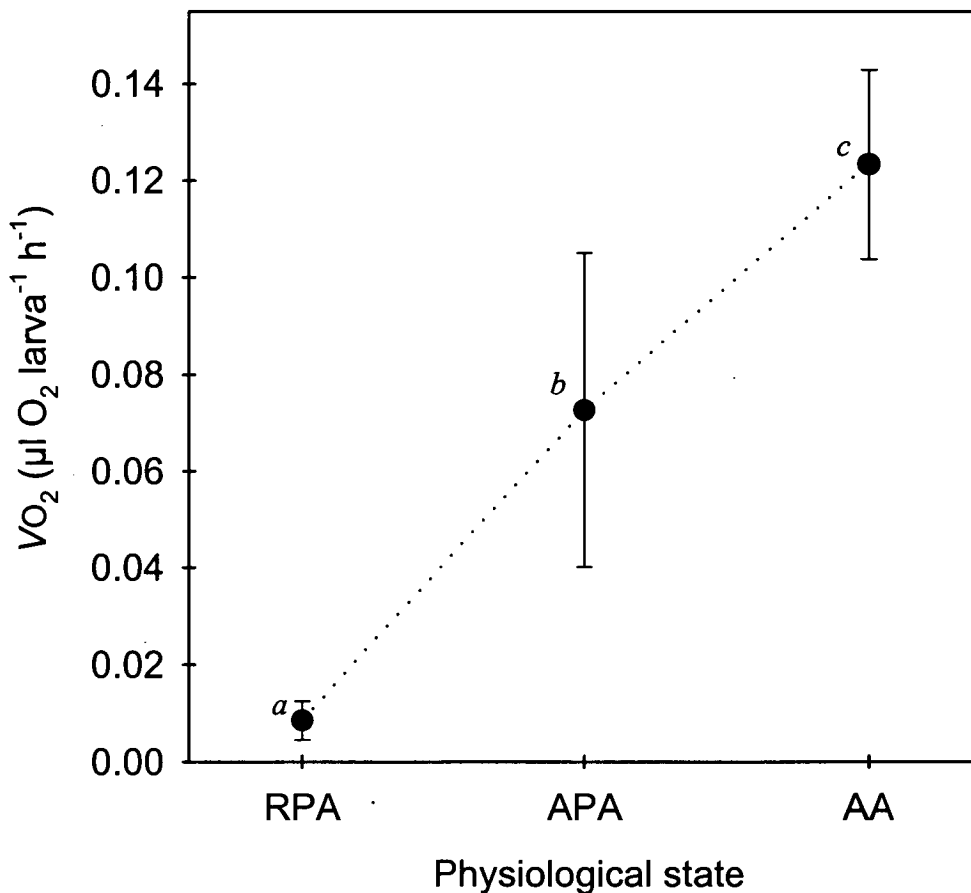


Figure 4.1 Mean (\pm SD) oxygen consumption by stage I *Jasus edwardsii* larvae under three different physiological states: (1) RPA (resting post-absorptive); (2) APA (active post-absorptive); and (3) AA (active absorptive). Treatments with different letters differed significantly (N-K, $P < 0.05$).

4.4.2 Experiment 1: The effect of temperature on the metabolic rate of resting and active larvae

The oxygen consumption of newly-hatched larvae was significantly dependent on their physiological state (2-ANOVA, $F_{1,24} = 59.85$, $P < 0.0001$; Fig. 4.6) and on temperature (2-ANOVA, $F_{2,24} = 76.81$, $P < 0.0001$). A significant interaction effect between temperature and the physiological state of the larvae was indicative of a change in response within the temperature range tested (2-ANOVA, $F_{2,24} = 18.85$, $P < 0.0001$). Respiratory rates increased in both resting and active larvae as temperature increased (Fig. 4.6). However, the effect of temperature was less pronounced in active larvae than in resting larvae as reflected in the differences in the Q_{10} values between animals under the two physiological states (Table 4.1). At 9.8 and 15.0°C, the aerobic metabolism of active larvae increased significantly ($P < 0.05$) from the resting rate, by factors of 5.4 and 2.1, respectively (Fig. 4.6). At 20.0°C, there was a change in the response of active larvae to temperature, and $\dot{V}O_2$ in resting and active larvae were not significantly different (Fig. 4.6; $P = 0.999$). The Q_{10} for 'cost of swimming' dropped from 0.93 in the range 9.8-15.0°C down to 0.02 in the range 15.0-20.0°C (Table 4.1).

4.4.3 Experiment 2: The effect of temperature on geotaxis

Temperature significantly affected the geotactic response of newly-hatched larvae ($P < 0.0001$; Fig. 4.7). Larvae were negatively geotactic at 10.0 and 15.0°C and became positively geotactic at 20.1°C.

4.4.4 Experiment 3: The effect of temperature on the rate of ascent of newly-hatched larvae

There was no difference between the rate of ascent of larvae at 9.9°C and at 14.9°C ($P = 0.888$; Table 4.1). It was not possible to measure the rate of ascent of larvae at 20.0°C which interestingly for newly-hatched animals displayed negative phototaxis (see Chapter 3) during the running of the experimental protocol.

Table 4.2 The effect of temperature on the oxygen consumption (V_{O_2}), Q_{10} , and ascent rates of newly-hatched *Jasus edwardsii* larvae anaesthetised or active. Temperatures are rounded up to the nearest integer.

Temperature	10°C	15°C	20°C
Resting rate			
V_{O_2} ($\mu\text{l O}_2\cdot\text{larva}^{-1}\cdot\text{h}^{-1}$)	0.0083 ± 0.0033	0.0331 ± 0.0084	0.0928 ± 0.0258
Q_{10}	14.3	7.7	
Active rate			
V_{O_2} ($\mu\text{l O}_2\cdot\text{larva}^{-1}\cdot\text{h}^{-1}$)	0.0449 ± 0.0163	0.0684 ± 0.0106	0.0976 ± 0.0154
Q_{10}	2.2	2.0	
Cost of swimming			
V_{O_2} ($\mu\text{l O}_2\cdot\text{larva}^{-1}\cdot\text{h}^{-1}$)	0.0366	0.0353	0.0048
Q_{10}	0.93	0.02	
Rate of ascent ($\text{cm}\cdot\text{s}^{-1}$)	0.43 ± 0.24	0.42 ± 0.23	—*

* This experiment could not be conducted at 20°C.

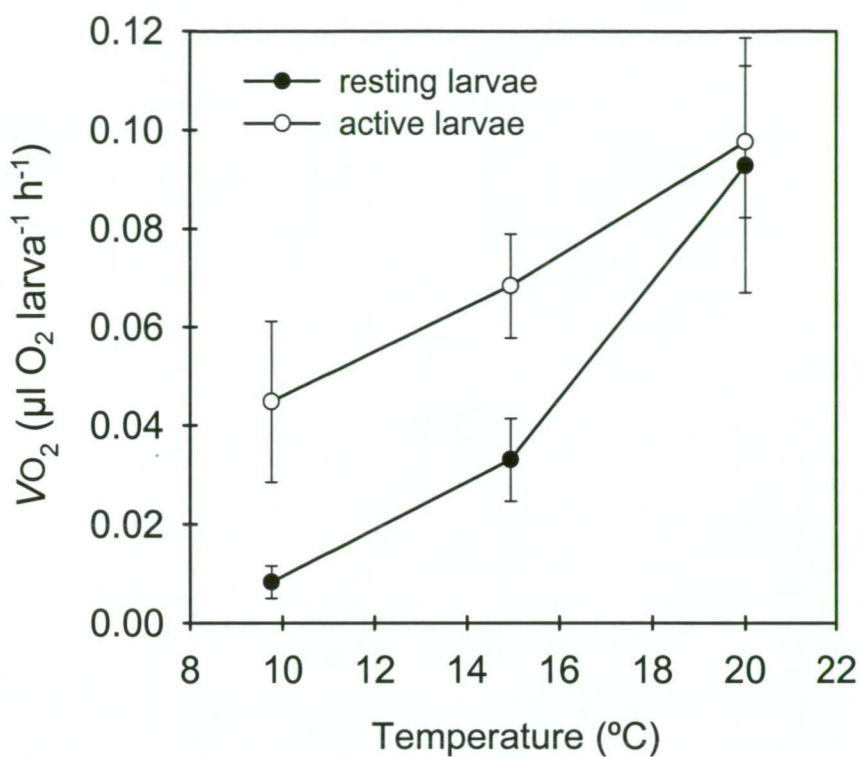


Figure 4.2 Mean (\pm SD) oxygen consumption by resting and active newly-hatched *Jasus edwardsii* larvae at three different temperatures.

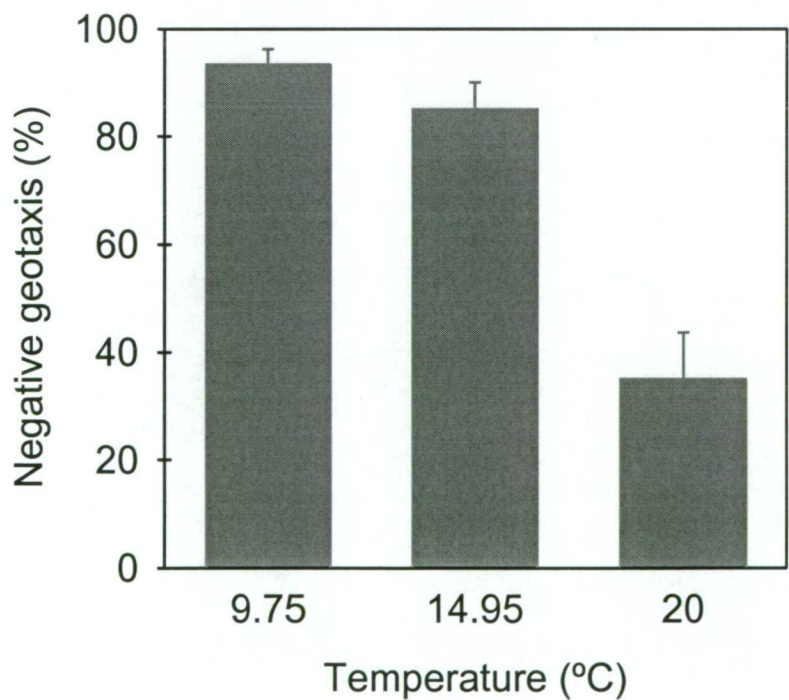


Figure 4.3 Mean (\pm SD) geotaxis in newly-hatched *Jasus edwardsii* larvae placed at three different temperatures.

4.5 Discussion

4.5.1 *The partitioning of metabolism*

Preliminary results demonstrated that the metabolic rate of *J. edwardsii* phyllosomas could be partitioned by manipulating feed intake and swimming activity. Clarke (1987) reviewed the metabolic processes that may contribute to the standard (SMR) and the routine metabolic rate (RMR) measured during respiration experiments on marine organisms. According to Clarke (1987), the difference in oxygen consumption between fed and starved larvae would represent the 'specific dynamic action' (SDA) which has been observed in several other planktonic species (see review by Raymont, 1983). The post-prandial metabolic pulse of fed animals, or SDA, reflects the cost of growth (biosynthesis) rather than the cost of feeding (gut activity, amino-acid oxidation and urea excretion) (Jobling, 1983; Kiørboe *et al.*, 1985; Thor, 2000). Therefore, SDA may be a valuable performance indicator in future work on the environmental and nutritional requirements of *J. edwardsii* larvae.

Standard metabolic rate has been measured or estimated in many aquatic organisms (Halcrow and Boyd, 1967; Vlymen, 1970; Klyashtorin and Yarzhombek, 1973; Klyashtorin and Kuz'micheva, 1976; Torres and Childress, 1983). However, anaesthesia has rarely been used to immobilise animals in order to perform direct measurements of SMR. The technique was reliable in estimating the standard metabolic rate of little skate (*Raja erinacea*) (Hove and Moss, 1997). Following the successful chemical immobilisation of stage I *J. edwardsii* larvae and the expedient way in which their metabolic rate could be measured in this resting state, the present study aimed at assessing whether or not anaesthesia may be a suitable to directly measure SMR in phyllosoma.

4.5.2 *Assessment of anaesthesia to estimate SMR*

4.5.2.1 *Response to external stimulus under anaesthesia*

J. edwardsii larvae treated with 2-phenoxyethanol showed sensitivity to external stimulus (i.e. temperature). This response to an external stimulus together with the presence of a stable heart beat rate for up to 6 h under 2-phenoxyethanol, indicated the operation of basal neurocircuitry, muscle tonus and movement of respiratory apparatus, processes which contribute to the standard metabolism as defined by Clarke (1983). Sensitivity to external stimulus under 2-phenoxyethanol was also reported in rainbow trout (Robb and Kestin, 1998).

4.5.2.2 *The energetic cost of swimming compared with behaviour*

Respirometry and visual observation techniques compared well in estimating the behavioural response to temperature of newly-hatched larvae. Although the metabolic rate of both resting and active larvae increased with temperature, the lower 'cost of swimming' at 20°C suggested a change in behaviour (reduced activity and possibly sinking) at elevated temperatures. This assumption was supported by positive geotaxis at 20°C (assumed to be due to reduced activity), while larvae exposed to 10 and 15°C were negatively geotactic and swam actively upwards. Therefore, according to respirometry and behavioural observations, the positive geotaxis reported at 20°C is likely to be due to the cessation of swimming activity that led to passive sinking. This phenomenon is common in decapod larvae which can regulate their depth distribution through changes in locomotor activity when faced with shifts in ambient temperature (see review by Sulkin, 1984). For instance, passive sinking at elevated temperatures was reported in *Rhithropanopeus harrisii* (Ott and Forward, 1976) and *Callinectes sapidus* (McConnaughey and Sulkin, 1984), while swimming activity declined in *Gammarus oceanicus* (Halcrow and Boyd, 1967), and in *Crangon crangon* (van Donk and de Wilde, 1981) exposed to high temperatures.

Furthermore, the level of swimming activity estimated from measurements of oxygen consumption agreed with the rates of ascent observed during vertical migration. Indeed, the similar rates of ascent recorded at 10 and 15°C corresponded with equivalent energetic cost of swimming at 10 and 15°C.

4.5.2.3 The cost of swimming assessed with anaesthesia

Under conditions stimulating swimming in spiny lobster larvae, the energy demand associated with swimming resulted in large increases in metabolic rate from a standard metabolic level (2.1 and 5.4 times at 10 and 15°C, respectively). Although these results were consistent with those obtained in *Gammarus oceanicus* (Halcrow and Boyd, 1967), *Gnathophausia ingens* (Mickel and Childress, 1978), *Euphausia pacifica* (Torres and Childress, 1983), and *Dioithona oculata* (Buskey, 1998), they disagreed with previous studies which found that energy expenditure for swimming was negligible compared to the overall energy expenditure of the animal (Vlymen, 1970; Klyashtorin and Yarzhombek, 1973; Foulds and Roff, 1976; Klyashtorin and Kuz'micheva, 1976). According to Torres and Childress (1983), these authors underestimated the energetic cost of swimming in planktonic crustaceans, chiefly because they reached their conclusion despite the failure to control activity when measuring standard metabolism.

An understanding of the effect of 2-phenoxyethanol on each of the components of standard metabolism (as defined by Clarke, 1983) remains beyond the scope of technical feasibility. However, the results obtained in the present study confirm the hypothesis that immobilisation with 2-phenoxyethanol may be used in estimating the SMR of *J. edwardsii* larvae. These results also support findings by Hove and Moss (1997) who used MS-222 to anaesthetise *Raja erinacea* in order to estimate diurnal changes in activity.

4.5.3 The Q_{10} of SMR, RMR, and 'cost of swimming'

The Q_{10} term, which is derived from the van't Hoff rule, is commonly applied by biologists to approximate the relationship between the rate of biological processes and temperature. Although declining with increasing temperature, the Q_{10} values obtained in resting larvae were unusually high (see review by Clarke 1983) suggesting a stress response and/or the absence of compensation for the standard metabolism of newly-hatched larvae. The stress possibly experienced by larvae at 10 and 20°C could be associated with the

limited period of acclimation (~ 1 h) to the experimental temperature for larvae acclimated to an incubation temperature near 15°C . The suppression of metabolism observed at 10°C could result from a lack of heat (Hochachka, 1990), whereas the overshoot observed in resting larvae at 20°C could be the consequence of an abrupt change in temperature (Prosser, 1973). Note, however, that the different metabolic processes that make up SMR are known to respond to temperature at different rates (Clarke, 1987). Therefore, measures of standard metabolic rate at two different temperatures (e.g. 10 and 20°C) may not necessarily be comparable and Q_{10} values must be interpreted cautiously.

In active larvae, Q_{10} values approaching 2 in the range 10 - 15°C and 15 - 20°C were consistent with the expected rate of chemical reaction of the van't Hoff rule. Based on the results stated above, it would be incorrect to interpret the consistency of the Q_{10} from 10 to 20°C as a linear relationship between metabolic response and temperature. Rather, and as indicated by findings on the metabolic rate of resting larvae and behaviour, the consistency of the Q_{10} in this instance, reflects a drop in activity together with the overshooting of the basal metabolic rate at 20°C . Values of the Q_{10} obtained for the 'cost of swimming' (near 1 and near 0 for the ranges 10 - 15°C and 15 - 20°C respectively) were consistent with behavioural response. Unless, the van't Hoff rule is applied to defined metabolic processes such as swimming activity and standard metabolism, the Q_{10} can potentially be misleading. Clarke (1983) noted that there was no logical basis for the application of the van't Hoff law to describe the metabolic response of whole organisms to temperature. Most importantly, we should stress the need to control or know the level of activity when assessing the effects of temperature on metabolism (Holeton, 1974, cited in Torres and Childress, 1983).

4.6 Conclusions

Both the avoidance behaviour and the lack of metabolic compensation at elevated temperature suggest that newly-hatched *J. edwardsii* larvae are behavioural thermoregulators. Measuring the metabolic rate of anaesthetised larvae was an effective method to determine standard metabolic rate and the energetic cost of swimming in newly-hatched *J. edwardsii* larvae. This technique may be used in future research to determine diurnal activity patterns (Hove and Moss, 1997) or to study the response of *J. edwardsii* larvae to environmental factors other than temperature. Additionally, the method described in the present study may be used to assess the behavioural response of aquatic organisms exposed to natural environmental conditions, which are often difficult to simulate *in situ*.

4.7 References

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Chapter 5

The effects of progressive
hypoxia on the oxygen
consumption of stage I
larvae at different
temperatures

5.1 Abstract

The effect of progressive hypoxia on oxygen consumption ($\dot{V}O_2$) was investigated in stage I *J. edwardsii* larvae at 14.2, 18.1 and 22.6°C to assess the tolerance of larvae to low oxygen tension and to determine whether their response was influenced by temperature. Under all temperatures tested, stage I larvae were partial oxyregulators as indicated by the curvilinear decline in $\dot{V}O_2$ under declining ambient dissolved oxygen (DO). Although the shape of the $\dot{V}O_2$ response pattern varied with temperature, P_{crit} (i.e. the critical oxygen tension at which $\dot{V}O_2$ is influenced by DO) remained unaffected and ranged from 3.90 to 4.32 ml O₂ l⁻¹. Recommendations for the rearing of phyllosoma were made on the basis of these critical levels of dissolved oxygen. The change in response pattern, particularly from 18.1 to 22.6°C indicated that the oxyregulatory capacity of stage I larvae increased at high temperature. This observation together with the significant decline in Q_{10} from the 14.2-18.1°C range to the 18.1-22.6°C range, implied thermal stress in larvae placed at 22.6°C.

5.2 Introduction

The respiratory response of animals faced with hypoxia is either to conform their oxygen consumption ($\dot{V}O_2$) to ambient oxygen (oxyconformers), or to regulate their $\dot{V}O_2$ (oxyregulators) so that $\dot{V}O_2$ is independent of progressive hypoxia (normoxic zone) down to a critical concentration of oxygen (DO_{crit}) below which $\dot{V}O_2$ becomes dependent upon further reduction of ambient dissolved oxygen (Vernberg and Vernberg, 1972; Willmer *et al.*, 2000). In marine invertebrates, the response to hypoxia is generally dictated by the dissolved oxygen variations inherent to their habitat (Vernberg and Vernberg, 1972). This was demonstrated by Ikeda (1977) who found a lower tolerance to hypoxia in zooplankters from oxygen rich surface waters than in organisms from oxygen-deficient bottom water. Crustaceans can nonetheless avoid anoxic or hypoxic conditions by moving to oxygen rich strata (Pihl *et al.*, 1991). However, such behaviour would have no significant benefit to organisms placed in a rearing tank where turbulence prevents oxygen stratification. From an aquaculture perspective, it is therefore essential to define the response pattern of *Jasus edwardsii* larvae to hypoxia (i.e. oxyconformity or oxyregulation) in order to determine water quality requirements and improve larval rearing practices.

This study aimed to determine the oxygen consumption response pattern and the critical value of dissolved oxygen in stage I *J. edwardsii* larvae subjected to progressive hypoxia. Since temperature may affect the oxyregulation capacity of invertebrates (Willmer *et al.*, 2000), the possible stress effect of increasing temperature was investigated through the assessment of changes in the respiratory response pattern to hypoxia of stage I *J. edwardsii* larvae acclimated to different temperatures (van Winkle and Mangum, 1975).

5.3 Material and methods

First stage *J. edwardsii* larvae were obtained throughout October and November 2000 from ovigerous females brought to the TAFI Marine Research Laboratories in July of the same year. Females were kept in indoor tanks on a flow-through system at ambient temperature ranging from 12.0 to 17.1°C, and fed regularly with squid and mussels until larval release. Newly-hatched larvae were collected at the surface of the tank and transferred into larval rearing units (Ritar, 2001). Prior to sampling for temperature acclimation, larvae were reared for three to four days on adult brine shrimps (1.5-2.5 mm) at 18°C. Acclimation to experimental temperature was conducted in static water 220 ml plastic jars placed floating in aquaria equipped with thermostats to maintain temperatures of 14, 18 and 22.5°C. The jars, filled with sea water and 25 ppm of oxytetracycline, were stocked with 20 larvae. Larvae were fed during acclimation (i.e. 1.5-2.5 mm adult *Artemia* to satiation) and feed was withdrawn from the jars at least 17 h prior to the start of the measurement of oxygen consumption. Therefore, $\dot{V}O_2$ was measured in post-absorptive larvae (see Appendix VI). $\dot{V}O_2$ under progressive hypoxia was assessed in 4, 5 and 6 d old larvae acclimated to 18, 22.5 and 14°C for 24, 48 and 48 h, respectively.

Changes in oxygen consumption under declining DO were assessed at 14.2 ± 0.1 , 18.1 ± 0.1 and $22.6 \pm 0.1^\circ\text{C}$ (mean \pm SD) by placing 12 larvae in a 2 ml respirometry chamber fitted above a polarographic oxygen electrode (Fig. 5.1), which was connected to a digital reader (Model 10, Rank Brothers Ltd, England). The larvae were acclimated to the experimental conditions for one hour during which oxygen saturated 0.2 μm filtered sea water treated with oxytetracycline (25 ppm) was passed through the chamber with a peristaltic pump. The flow of water was stopped to monitor the decline in oxygen content in the chamber with SmartReader 7 (ACR System Inc.) data loggers connected to the digital reader. The DO in the chamber was left to drop down to below 2 ml $\text{O}_2 \text{ l}^{-1}$. Larvae were then retrieved to determine their dry weight (as per method described in

Chapter 3). To estimate oxygen consumption by the electrode, the chamber was flushed with oxygen saturated seawater and the decline of DO was recorded overnight with a data logger. Despite a 1-h acclimation period, initial readings were elevated. Stable readings were estimated to be reached at 5.2, 4.8 and 4.4 ml l⁻¹ at 14.2, 18.1 and 22.6°C, respectively. These levels of DO (~90% saturation) were obtained within the first 30 min of recording suggesting that a period of acclimation of 1.5 h is required for stage I *J. edwardsii* larvae to recover from handling stress and reach a stable $\dot{V}O_2$. Data are presented as mean \pm SD μ l O₂ mg DW⁻¹ h⁻¹ of four trials at declining DO intervals of 0.4 ml O₂ l⁻¹, after deduction of background oxygen consumption by the electrode.

The relative degree of oxyconformity (i.e. linear decline of $\dot{V}O_2$ with decreasing DO) or oxyregulation (i.e. $\dot{V}O_2$ is constant over a range of DO) was evaluated by fitting quadratic regression models to the $\dot{V}O_2$ response to declining DO at each temperature (van Winkle and Mangum, 1975). The effects of temperature on the intercept and the shape of the quadratic relationship between $\dot{V}O_2$ and DO were tested with the Kimura Likelihood Ratio test (KLR) following the procedure described by Haddon (2001). $\dot{V}O_{2\max}$ (i.e. level of the maximum $\dot{V}O_2$ under progressive hypoxia) and DO_{crit} (i.e. critical oxygen concentration at which $\dot{V}O_2$ becomes DO dependent) were determined for larvae from each brood and at each temperature by fitting a segmented model with plateau to the response curve. This analysis was conducted in SAS using the NLIN procedure (SAS Institute, 1990). The effect of temperature on $\dot{V}O_{2\max}$ and DO_{crit} was assessed with analysis of variance (ANOVA) followed by the Least Significant Difference test (LSD) for multiple comparison. The Q₁₀ was computed using $\dot{V}O_{2\max}$ data according to the formula cited in Chapter 3. The possible change in Q₁₀ between the 14.2-18.1°C range and the 18.1-22.6°C range was analysed with a t-test. ANOVA, t-test and LSD were conducted in JMP 3.1 statistical software.

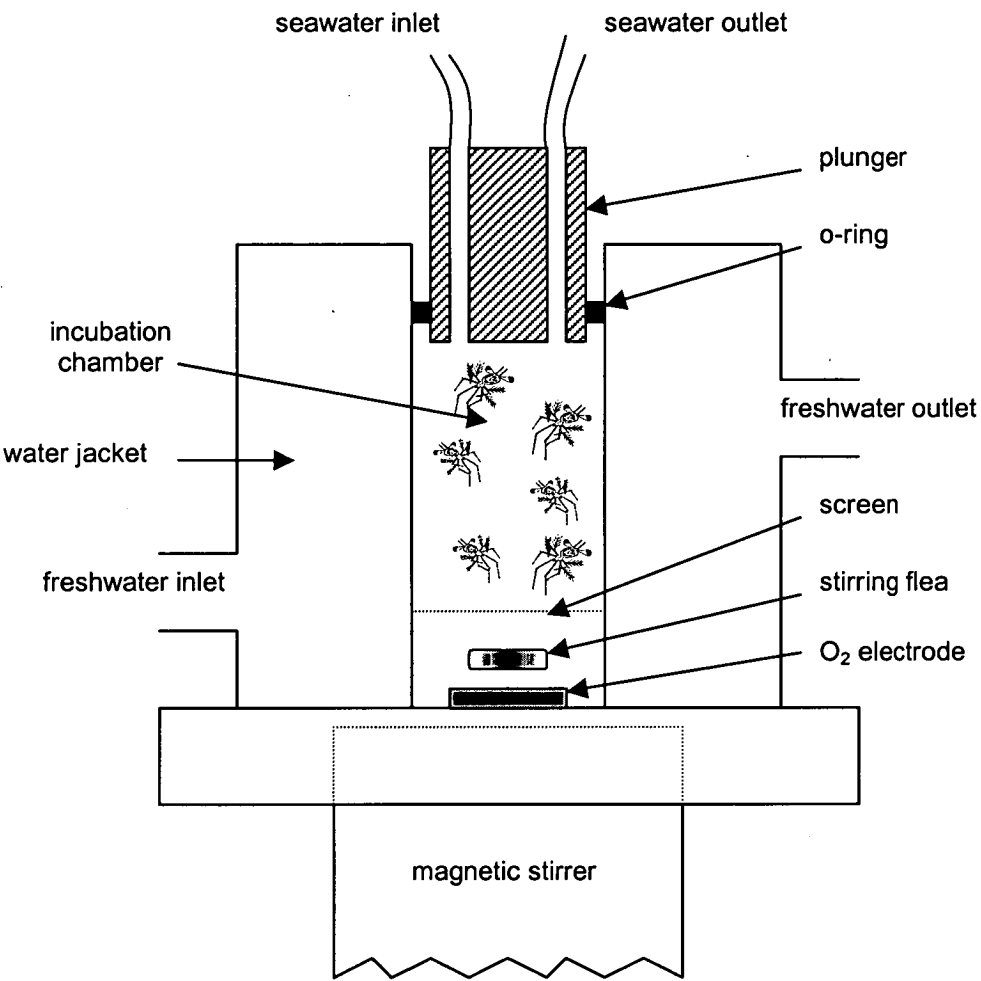


Figure 5.1 Schematic view of the experimental apparatus used to measure the oxygen consumption of stage I *Jasus edwardsii* larvae subjected to declining DO at different temperatures.

5.4 Results

Quadratic models illustrated the significant decline of $\dot{V}O_2$ by larvae exposed to declining DO (Fig. 5.2) at 14.2°C ($r^2 = 0.48$, $F_{2,129} = 58.61$, $P < 0.0001$), 18.1°C ($r^2 = 0.60$, $F_{2,113} = 85.43$, $P < 0.0001$), and 22.6°C ($r^2 = 0.63$, $F_{2,97} = 81.16$, $P < 0.0001$). $\dot{V}O_{2\max}$ was temperature dependent (ANOVA, $F_{2,9} = 26.17$, $P < 0.001$) and significantly increased from 14.2 to 18.1°C and again from 18.1 to 22.6°C (Table 5.1). Nonetheless, according to the Q_{10} , the change in $\dot{V}O_{2\max}$ was greater between 14.2 and 18.1°C than between 18.1 and 22.6°C (t-test, $F_{1,6} = 6.72$, $P < 0.05$, Table 5.1). Temperature did not affect the DO_{crit} of stage I larvae (ANOVA, $F_{2,9} = 0.12$, $P = 0.887$, Table 5.1).

As indicated by the Kimura Likelihood Ratio test, there was a change in the $\dot{V}O_2$ response to declining DO over the range of temperatures tested (KLR on β_1 and β_2 , $df = 2$, $\chi^2 = 28.18$, $P < 0.0001$). Pairwise comparison of models between temperatures showed a significant change in the shape (coefficients β_1 and β_2) of the metabolic response to progressive hypoxia from 18.1°C to 22.6°C (Table 5.2). The significant decline of the β_2 coefficient at 22.6°C (Table 5.2) suggests increased oxyregulatory capacity in stage I larvae subjected to high temperature.

Table 5.2 Mean (\pm SD) $\dot{V}O_{2\max}$ ($\mu\text{l O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$), Q_{10} and DO_{crit} ($\text{ml O}_2 \text{ l}^{-1}$) in stage I *Jasus edwardsii* larvae exposed to three different acclimation temperatures. Values within each row bearing different superscripts were significantly different (LSD, $P < 0.05$).

temperature (°C)	14.2	18.1	22.6
$\dot{V}O_{2\max}$	0.68 ± 0.07^a	0.87 ± 0.08^b	1.01 ± 0.04^c
Q_{10}		1.89 ± 0.27^a	1.42 ± 0.24^b
DO_{crit}	4.30 ± 1.51	4.32 ± 1.03	3.90 ± 1.49

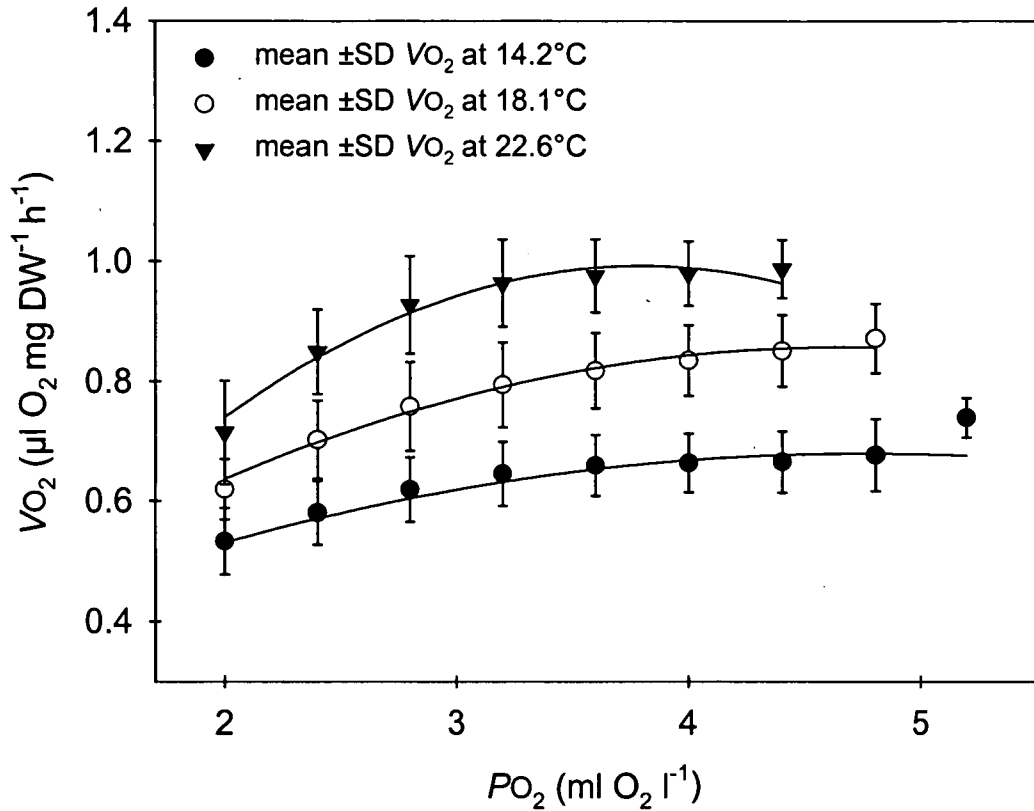


Figure 5.2 Respiratory response of stage I *Jasus edwardsii* larvae under declining DO at three different temperatures. Response at each temperature was fitted with a quadratic polynomial model (Table 5.2).

Table 5.2 Coefficient estimates and their significance for quadratic polynomial models fitted to the respiratory response of stage I *Jasus edwardsii* larvae under declining DO at three different temperatures (Fig. 5.2). Coefficients of a same row with different superscripts differed significantly (KLR, $P < 0.05$).

	14.2°C		18.1°C		22.6°C	
Coefficient	Estimate	P	Estimate	P	Estimate	P
β_0	0.2371 ^a	<0.001	0.1784 ^a	0.060	-0.1370 ^b	0.290
β_1	0.1855 ^a	<0.0001	0.2913 ^a	<0.0001	0.5947 ^b	<0.0001
β_2	-0.0195 ^a	<0.001	-0.0313 ^a	<0.001	-0.0783 ^b	<0.0001

5.5 Discussion and conclusions

The curvilinear $\dot{V}O_2$ response pattern to progressive hypoxia under three different temperatures indicated partial oxyregulation in stage I *J. edwardsii* larvae (van Winkle and Mangum, 1975). However, given the high DO_{crit} observed in phyllosomas, their capacity for oxyregulation appeared to be relatively limited compared to *Carcinus maenas* (Taylor *et al.*, 1977) and *Penaeus monodon* (Liao and Murai, 1986). In their natural coastal habitat both *C. maenas* and *P. monodon* may frequently be faced with hypoxic conditions in contrast with *J. edwardsii* larvae which live in a well oxygenated oceanic habitat with little fluctuation in dissolved oxygen (Willmer *et al.*, 2000). For instance, depth oxygen profiles recorded in waters off the northeastern coast of Tasmanian (150°E, 40°S) indicated minimum dissolved oxygen concentration in the summer of 5.1 and 4.6 ml O_2 l⁻¹ at the surface and at 100 m depth, respectively (Jeff Dunn, personal communication; data extracted from the CSIRO Atlas of Regional Seas database). Therefore, the partial oxyregulation observed in stage I *J. edwardsii* larvae is not surprising, considering the relative consistency in dissolved oxygen encountered in their habitat. This hypothesis is in agreement with Belman and Childress (1973) who, discussing the limited oxygen regulatory capabilities of *Panulirus interruptus* stage I larvae, concluded on the inappropriateness of mechanisms for oxygen regulation in organisms occurring at the surface of the ocean. Ikeda (1977) provided additional evidence to this argument when he found that the effect of lowered oxygen concentration was less pronounced in zooplankters collected from oxygen-deficient bottom water, than in species occurring in oxygen-rich surface waters. It is therefore possible that given their oceanic habitat *J. edwardsii* larvae would not be adapted to low oxygen tension. This could be intuitively supported by the development of gills at the 11th and last larval stage (Lesser, 1978), before metamorphosis into the puerulus that will assume benthic settlement.

Although, the DO_{crit} of stage I *J. edwardsii* larvae did not change with acclimation temperature, the β_2 coefficient of the quadratic regression describing

the effect of progressive hypoxia was lower at 22.6°C than at 14.2 and 18.1°C. According to van Winkle and Mangum (1975) this implies increased oxyregulation in *J. edwardsii* larvae exposed to higher temperatures. These results clearly contrast with the general trend reported in crustaceans and teleosts for which the oxyregulating capacity declines under raising temperature (Taylor *et al.*, 1977; Bridges and Brand, 1980; Schurmann and Steffensen, 1997; Claireaux and Lagardère, 1999). For instance, the increase in DO_{crit} with increasing temperature that was reported in *Carcinus maenas*, was attributed to the decline in oxygen affinity of blood pigments that is associated with raising temperature (Taylor *et al.* 1977). In addition, intrinsic factors such as activity that may be influenced by temperature can affect the response of aquatic organisms to hypoxia. Bridges and Brand (1980), and Claireaux and Lagardère (1999) demonstrated that higher levels of activity profoundly reduced the capacity for oxyregulation in *Galathea strigosa*, and *Dicentrarchus labrax*, respectively. Even though the level of activity was not assessed in the present study, observations of behaviour reported in Chapter 3 and Chapter 4 indicated a marked reduction of locomotor activity in newly-hatched and mid-stage I *J. edwardsii* larvae subjected to elevated temperatures. Therefore, an increase in the level of activity of larvae at 14.2 and 18.1°C may explain the reduced oxyregulatory response observed under these conditions compared with larvae exposed to 22.6°C. Furthermore, the marked decline in the β_2 coefficient of the quadratic regression at 22.6°C implies a stress response under such conditions as suggested by van Winkle and Mangum (1975). This is supported by a decline in the Q_{10} from the 14.2-18.1°C range to the 18.1-22.6°C range, a change in Q_{10} being indicative of a shift in response (Vernberg and Vernberg, 1972).

The oxygen affinity of haemocyanin is known to decline at higher temperature, and at lower pH according to the normal Bohr shift (Mangum and Ricci, 1989; Willmer *et al.*, 2000). Therefore, the similar $\dot{V}O_2$ response under progressive hypoxia observed in *J. edwardsii* larvae at 14.2 and 18.1°C implies the contribution of an underlying compensatory mechanism at 18.1°C. Considering the absence of gills in the first 10 larval stages and the flattened morphology of the phyllosoma (Lesser, 1978), diffusion through the body surface

seems a likely pathway for gas exchange. Consequently, under conditions causing lower oxygen affinity in blood pigments (i.e. high temperatures) greater efficiency in oxygen uptake could be achieved by increasing ventilation in order to enhance the gradient of O_2 partial pressure between extrinsic and intrinsic oxygen tensions. External ventilation at the body surface may be achieved through locomotor activity or the beating of setae. However, the higher metabolic demand that would result from such a mechanism was not observed in the present study. Alternatively, increased ventilation could be achieved intrinsically through enhanced cardiac output and therefore, enhanced perfusion rate. Heart beat rate being temperature dependent in invertebrates (deFur and Mangum, 1979) the expected higher cardiac output of phyllosoma at 18.1°C could be a sufficient mechanism to compensate for lower oxygen blood affinity and achieve temperature independence of the oxyregulatory capacity in *J. edwardsii* larvae.

Although stage I *J. edwardsii* larvae appeared to be partial oxyregulators, they are not well adapted to sustain hypoxia in culture and dissolved oxygen levels should be kept above $4.3\text{ ml } O_2\text{ l}^{-1}$ ($\sim 6.15\text{ mg } O_2\text{ l}^{-1}$). The concentration recommended here is greater than the minimum safe dissolved oxygen concentration (i.e. $5\text{ mg } O_2\text{ l}^{-1}$) generally prescribed in aquaculture manuals (Forteath, 1990) and textbooks (Barnabé, 1991). Therefore, special care must be taken in the design of a rearing system to insure that oxygenating devices are installed. In intensive systems, dissolved oxygen levels should also be monitored regularly and particularly after feeding events given the post-prandial rise in oxygen consumption reported in Chapter 4.

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Chapter 6

The effects of changes in
ambient salinity on the
behaviour, growth, and
metabolic rate
of stage I larvae

6.1 Abstract

This study aimed at defining the effects of fluctuating salinity on *Jasus edwardsii* larvae. The effects of sudden changes in salinity from ambient conditions at hatching (i.e. 34.5 ppt) to 25, 28, 31, 34 and 37 ppt were examined through investigations of behavioural and respiratory responses in newly-hatched larvae. Although a linear increase in negative geotaxis with increasing salinity indicated a preference for higher salinities, the interpretation of the respiratory response remained ambiguous due to the compounded effects of salinity on locomotor activity and buoyancy. In a second experiment larvae were reared from hatching through to stage II under continuous salinity regimes (C) at 28, 31, 34 and 37 ppt and under repeated exposure (R) to 28, 31 and 37 ppt from a control salinity of 34 ppt. Continuous exposure to 28, 31 and 37 ppt and repeated exposure to 31 and 37 ppt did not delay development as opposed to the repeated exposure to 28 ppt. While post-moult growth to stage II was not suppressed by fluctuating salinity in the 31R and 37R ppt groups, larvae were significantly smaller in the 28R, 28C, 31C and 37C ppt regimes than in the 34 ppt continuous control treatment. The effect of salinity acclimation on the respiratory response of mid-stage I larvae was examined in a third experiment. The increased metabolic rate of non-acclimated larvae at reduced salinities reflected the energetic cost associated with osmotic stress and with regular shifts in ambient salinity. However, the decline in oxygen consumption in larvae acclimated to a salinity of 28 ppt did not support the assumption that reduced growth under continuous osmotic stress may be linked to the metabolic cost of osmoregulation. The results of the present study were discussed with respect to the physiological cost of cell volume regulation in animals subjected to changes in ambient salinity.

6.2 Introduction

The effect of salinity on marine invertebrates is well documented and ranges from sublethal to lethal according to the magnitude of the change in salinity and the tolerance capacity of the species. Estuarine and coastal crustaceans are often euryhaline and can withstand large shifts in external salinity as opposed to their stenohaline oceanic counterparts that live in or can actively select isohaline waters (Willmer *et al.*, 2000). In a euryhaline species such as *Carcinus maenas* for instance, larval development is not affected by salinities ranging from 25 to 32 ppt (Anger *et al.*, 1998). In contrast, the stenohaline *Pandalus borealis*, known to have an optimal salinity around 31 ppt, does not complete larval development at 25 ppt (Wienberg, 1982). In culture situations, sublethal effects of salinity stress such as delayed development and reduced growth (Anger *et al.*, 1998; Pechenik *et al.*, 2000; Hereu and Calazans, 2000; Kumlu *et al.*, 2001) may reflect the energetic costs associated with specific avoidance behaviours (Scarratt and Raine, 1967; Latz and Forward, 1977) or cell volume regulation (Hawkins and Hilbish, 1992; Lankford and Targett, 1994; Woo *et al.*, 1997).

Since most of the larval development of panilurid species takes place in oceanic waters (Phillips and McWilliam, 1986) of relatively constant salinity, larvae of these species are not expected to have the capacities to withstand the large shifts in ambient salinity that can occur in coastal and estuarine areas where marine hatcheries are often located. For instance, salinities ranging from 29.5 to 34.5 ppt have been recorded for the incoming water at the marine laboratories of the Tasmanian Aquaculture and Fisheries Institute located on the Derwent estuary (Tasmania, Australia) during a two month period during the summer of 1996 (Chamchang, 1997). The present work was carried out to examine the effect of changes in ambient salinity on the behaviour, survival, growth and metabolic rate of the first stage of the spiny lobster, *Jasus edwardsii*. The experimental salinities cover the range of salinities reported in coastal waters of Tasmania where research on the propagation of this species is being conducted. The range was also

extended to supranormal salinities that can occur in hatcheries working with a recirculation system. The effects of salinity on the geotaxis and oxygen consumption of the positively phototactic newly-hatched larvae were first assessed in an attempt to determine the origin of energy expenditure at different salinities. More animals were reared at different salinities throughout stage I and the effect of frequent changes in salinity was investigated through repeated exposure to sub and supranormal concentrations. In order to assess the link between the effect of salinity on development, and the metabolic response to changes in ambient salinity, oxygen consumption was measured in correspondingly acclimatised (continuous exposure) and non-acclimatised (repeated exposure) larvae.

6.3 Material and methods

6.3.1 *Origin of larvae*

Larvae were sourced from ovigerous females caught off the east coast of Tasmania from June to October in 1999 and 2000, and brought to the TAFI Marine Research Laboratories (Hobart, Australia). Newly-hatched larvae were collected from hatching tanks from September to December of both years. The ambient salinity at the time of hatching ranged from 31.2 to 35.3 ppt.

6.3.2 *Behaviour and physiology of newly-hatched larvae*

6.3.2.1 Behaviour

The geotaxis of newly-hatched larvae was examined in seawater of different salinities. Waters at salinities ranging from 25 to 37 ppt with a three ppt increment (25, 28, 31, 34 and 37 ppt) were obtained by mixing 0.2 μm filtered seawater with deionised freshwater or hypersaline water (~ 40 ppt). Hypersaline water was obtained by heating ($\sim 40^\circ\text{C}$) filtered seawater for a few days. Salinity was monitored with a YSI Model 63 salinity meter during mixing. At each salinity, 20 newly-hatched larvae were placed in each of three glass tubes with a flat bottom (H: 300 mm, \varnothing : 30 mm). The position of the larvae in the column (top or bottom half of the tube) was recorded after 30 min exposure. Geotaxis was observed in a total of 1200 larvae from four broods. Larvae were gradually acclimatised to the experimental temperature (18°C) over a period of 2 h. Data are expressed as mean ($\pm\text{SE}$) negative geotaxis between progenies.

The loss in buoyancy associated with decreasing salinity was assessed in newly-hatched larvae narcotised for at least 30 min in a solution of seawater and 2-phenoxy-ethanol (1 ml l^{-1}). The sinking of newly-hatched larvae over a vertical drop of 10 cm in mid-water was timed in a glass vertical column with a dark background. Twenty larvae from each of three broods were examined at each salinity (25, 28, 31, 34 and 37 ppt). Data are expressed as mean ($\pm\text{SE}$) sinking rate between broods.

6.3.2.2 Physiology

The oxygen consumption ($\dot{V}O_2$) of newly-hatched larvae was measured at salinities of 25, 28, 31, 34 and 37 ppt. The experiment was repeated on two different broods. Oxygen uptake was measured at each salinity in five replicate respirometers, which contained 10 larvae each, and two control respirometers without larvae. The syringe respirometers used in the present study were described earlier in Chapter 3 (Fig. 3.2.A). Oxytetracycline (25 ppm; Engemycin 100, Intervet) was added to the water in the respirometers to reduce microbial background respiration. Oxygen consumption was measured for 5-6 hours after 1-2 h of recovery from handling, and was expressed in $\mu\text{l O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$ following the experimental protocol described in Chapter 3. Data obtained from the broods tested were pooled for analysis and expressed as mean (\pm SD).

6.3.3 *Acclimation in stage I larvae*

6.3.3.1 Rearing at different salinities

To examine the effect of frequent changes in ambient salinity (28, 31, 34 and 37 ppt) on the survival and growth of stage I larvae, newly-hatched animals were placed under two salinity regimes: continuous exposure (C) and repeated exposure from a control salinity level (R). In the repeated exposure group, larvae were exposed to their respective treatment salinity (28, 31 or 37 ppt) at the start of the trial. After 24 h of exposure to these conditions, larvae were returned to the 'normal' control salinity (34 ppt) for 24 h before a further 24 h exposure to treatment salinity, and so on. In both continuous and repeated exposure groups, larvae were returned to the control salinity from day 9 after hatching and until they moulted into stage II so that all larvae would moult under the same conditions. During the intermoult period, larvae in the repeated exposure group were placed five times of 24 h each under treatment salinities. Larvae were reared in 60 ml plastic jars with 50 ml of water and 25 ppm of oxytetracycline. There were 12 larvae per jar (4 from each of 3 broods) and five replicated jars per treatment. Feeding of live *Artemia* (1-2 mm in length), complete water exchange (+25 ppm oxytetracycline), and removal of dead larvae were carried out daily. All larvae that moulted into stage II were measured for total body length (from the

anterior of the cephalic shield to the end of the telson) on a Nikon Profile Projector Model 6C to the nearest 25 μm . Minimum (after water exchange) and maximum (before water exchange) salinity were recorded daily and averaged to obtain a salinity measurement for each day during rearing. Mean ($\pm\text{SD}$) salinity levels were 28.05 ± 0.14 , 31.06 ± 0.08 , 34.09 ± 0.15 , 37.08 ± 0.17 ppt and are rounded up to the nearest integer in the text. The pH at each salinity was initially adjusted to 8.2 with a few drops of a sodium bicarbonate solution (pH 9). The mean ($\pm\text{SD}$) pH in culture media from daily measurements of initial (after water exchange) and final pH (before water exchange) were 8.01 ± 0.04 , 8.02 ± 0.04 , 8.04 ± 0.05 and 8.07 ± 0.05 at 28, 31, 34 and 37 ppt, respectively. The pH was not significantly different between salinity treatments (ANOVA, $F_{3,32} = 2.85$, $P > 0.05$). Mean ($\pm\text{SD}$; daily measurement) rearing temperature was $18.53 \pm 0.23^\circ\text{C}$.

6.3.3.2 Routine metabolic rate

The effect of acclimation to sub- and supra-normal salinities on the routine metabolic rate (RMR: oxygen consumption in unrestrained animals) of stage I larvae was determined from measurements of $\dot{V}\text{O}_2$ at different salinities (28, 31, 34 and 37 ppt) and in two groups of larvae: acclimated (A) and non-acclimated (NA). Acclimated larvae were 4-day-old animals reared from hatching at constant salinities (mean $\pm\text{SD}$, $n = 3$) of 28.6 ± 0.5 , 31.7 ± 0.6 , and 37.6 ± 0.2 ppt. Non-acclimated larvae of the same age had been cultured at the control salinity (mean $\pm\text{SD}$, $n = 3$) of 34.7 ± 0.4 ppt. Larvae were reared to day 4 in 60 ml plastic jars with 50 ml of seawater and 25 ppm of oxytetracycline. Feeding and water exchange were carried out daily. There were 10 larvae per jar and animals from each jar were placed in a respirometer. Larvae were sourced from three broods. There were six replicate respirometers (two for each brood) and two control respirometers per treatment. Total animal dry weight (method detailed in Chapter 3) was determined in all respirometers and oxygen consumption was expressed in $\mu\text{l O}_2 \text{ mg DW}^{-1} \text{ h}^{-1}$ following the experimental protocol described in Chapter 3. The minimum final DO observed in respirometers throughout the present trials (i.e. 4.1 ml l^{-1}) was close to the DO_{crit} (i.e. 4.3 ml l^{-1}) determined for stage I larvae (see Chapter 5). Data were expressed as mean ($\pm\text{SD}$).

6.3.4 *Statistical analysis*

All data were tested for normality (Shapiro-Wilk W test) and homoscedasticity (Levene's test or regression analysis of standard deviation against mean). Linear regression analyses were applied to the geotaxis and the sinking rate of newly-hatched larvae in response to salinity. The effect of salinity on the oxygen consumption of newly-hatched larvae was analysed with one way analysis of variance (ANOVA) and multiple comparison was carried out with the least significant difference test (LSD). Two way analyses of variance (2-ANOVA) excluding the control group (34 ppt, continuous exposure or acclimated) were used to describe the combined effect of type of exposure and acclimation with salinity on survival, duration of intermoult period, post-moult body length and oxygen consumption. Analyses of variance were also performed on each exposure or acclimation group (continuous vs. repeated and acclimated vs. non-acclimated) and treatments at subnormal salinities or with a different acclimation or exposure regime were compared to the control group (34 ppt, continuous exposure or acclimated) with the Dunnett's test. T-tests were used to highlight specific differences between groups or treatments that were not detected by the above statistics. Survival data were arcsine square root transformed before analysis. All computations were carried out with JMP 3.1 statistical software.

6.4 Results

6.4.1 *Behaviour and physiology of newly-hatched larvae*

6.4.1.1 Behaviour

The occurrence of negatively geotactic larvae increased linearly with increased salinity (linear regression, $r^2 = 0.580$, $F_{1,18} = 23.52$, $P < 0.001$; Fig. 6.1), while the rate of sinking of narcotised larvae declined linearly with increasing salinity (linear regression, $r^2 = 0.567$, $F_{1,13} = 17.03$, $P < 0.01$; Fig. 6.2).

6.4.1.2 Routine metabolic rate (RMR)

Salinity had a significant effect on the respiratory rates measured in newly-hatched larvae (ANOVA, $F_{4,64} = 13.77$, $P < 0.0001$; Fig. 6.3). Oxygen consumption was highest in animals at 31 ppt (LSD, $P < 0.05$) and from this salinity oxygen consumption declined as larvae were exposed to lower and higher salinities (Fig. 6.3). The least oxygen consumption was observed in larvae at 37 ppt (LSD, $P < 0.05$) and oxygen uptake in larvae at 25 ppt was not significantly different than at 28 ppt and 34 ppt (LSD, $P > 0.05$).

6.4.2 *Acclimation in stage I larvae*

6.4.2.1 Survival

Overall mean (\pm SD) survival of stage I larvae to stage II was $75.0 \pm 15.6\%$. Survival was uniform across salinities (2-ANOVA, $F_{2,24} = 0.28$, $P = 0.758$; Table 6.1) and was neither different between continuously exposed and repeatedly exposed larvae (2-ANOVA, $F_{1,24} = 1.36$, $P = 0.255$) nor was the response pattern different between the two exposure groups (2-ANOVA, salinity \times exposure, $F_{2,24} = 2.79$, $P = 0.082$; Table 6.1).

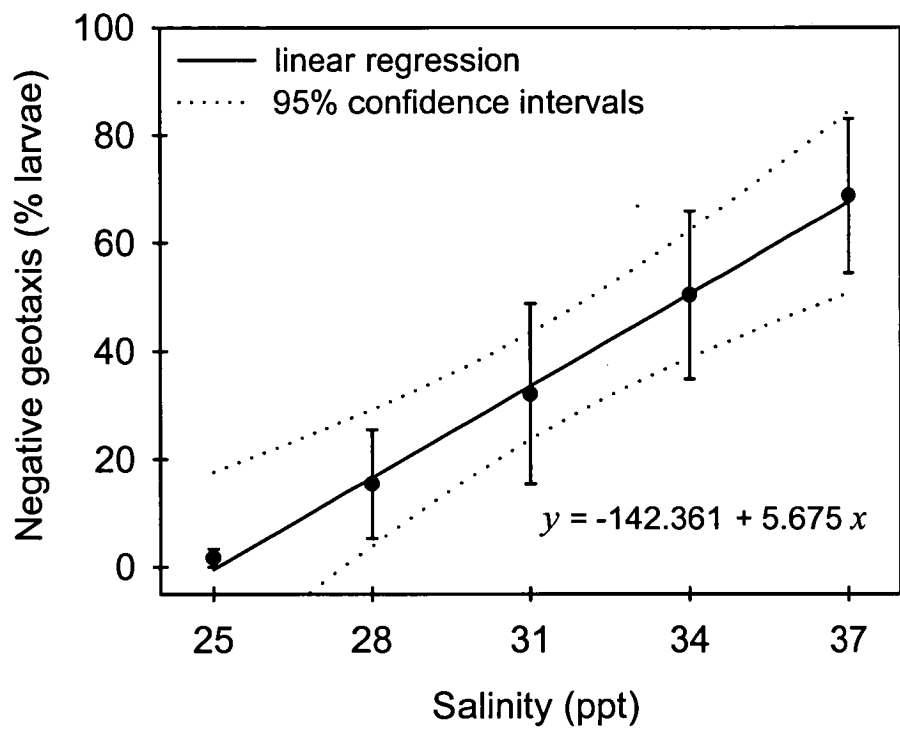


Figure 6.1 Linear relationship between salinity and the negative geotaxis displayed by newly-hatched *Jasus edwardsii* larvae. Mean (\pm SE), 95% confidence intervals and equation are shown.

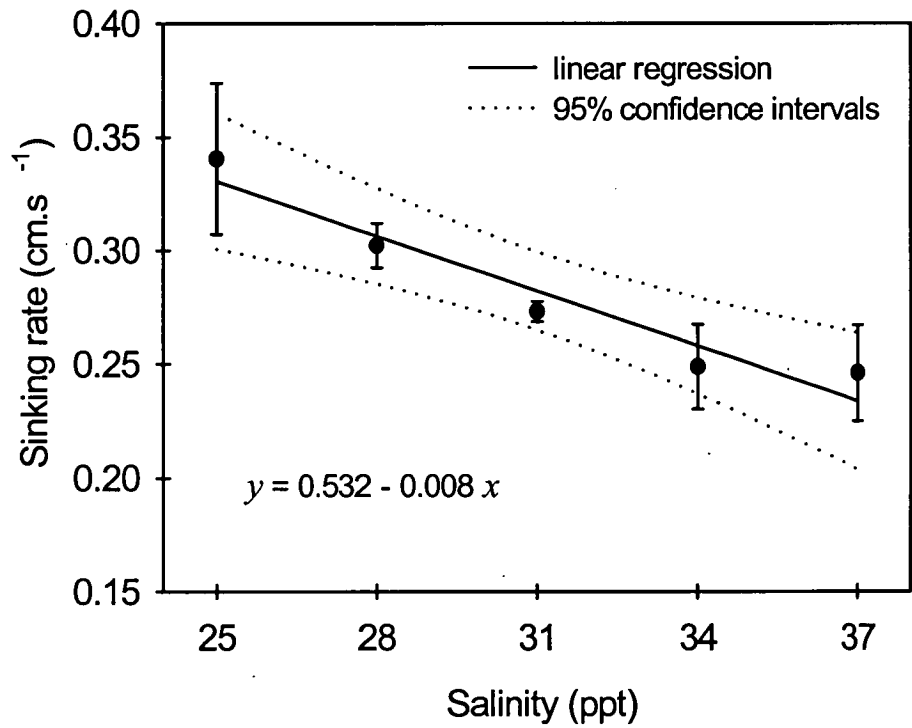


Figure 6.2 Linear relationship between sinking rate and salinity in newly-hatched *Jasus edwardsii* larvae. Mean (\pm SE), 95% confidence intervals and equation are shown.

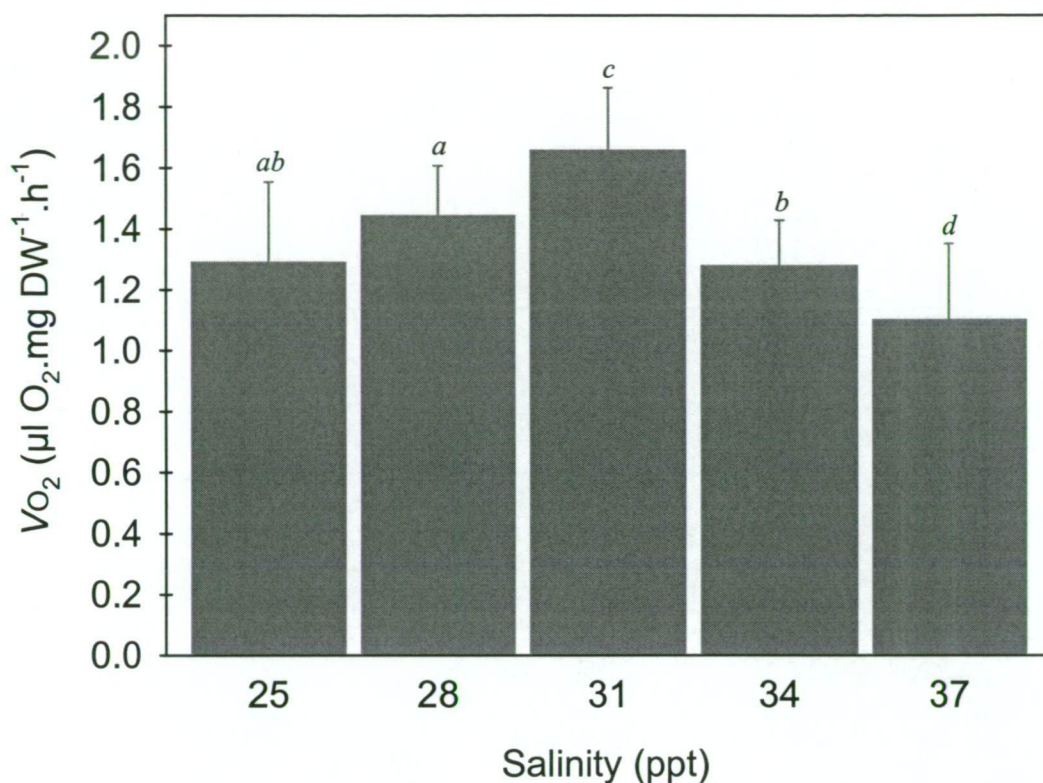


Figure 6.3 Mean (\pm SD) oxygen consumption in newly-hatched *Jasus edwardsii* larvae exposed to different salinities. Treatments with different letters are significantly different (LSD, $P < 0.05$).

Table 6.2 Post-moult percent survival (mean \pm SD) to stage II in *Jasus edwardsii* larvae reared either under continuous or repeated exposure to different treatment salinities.

Salinity (ppt)	Exposure	
	Continuous	Repeated
28	78.00 \pm 10.95	68.00 \pm 21.68
31	56.00 \pm 26.08	80.00 \pm 7.07
34	74.00 \pm 15.17	—
37	70.00 \pm 7.07	76.00 \pm 5.48

6.4.2.2 Intermoult period

Overall, the duration of the intermoult period was affected by salinity (2-ANOVA, $F_{2,24} = 6.53$, $P < 0.01$) in stage I *J. edwardsii* larvae (Fig. 6.4). While there was no significant difference in developmental time between larvae in the continuous and repeated exposure groups (2-ANOVA, $F_{1,24} = 0.16$, $P = 0.690$), the pattern of response to salinity was different between the two exposure groups (2-ANOVA, $F_{2,24} = 5.69$, $P < 0.01$). Indeed, continuous exposure to salinities ranging from 28 to 37 ppt had no effect on intermoult duration (ANOVA, $F_{3,16} = 2.90$, $P = 0.067$), whereas repeated exposure within the same range of salinities significantly affected development (ANOVA, $F_{2,12} = 8.16$, $P < 0.01$). Consequently, development was slower in larvae reared in 28R ppt than in larvae at 34C ppt (Dunnett's, $P < 0.05$; Fig. 6.4). The difference in response between continuous and repeated exposure groups was particularly marked at 37 ppt (t-test, $t_8 = 10.73$, $P < 0.05$; Fig. 6.4).

6.4.2.3 Growth

Salinity significantly affected body length growth in both continuous exposure (ANOVA, $F_{3,16} = 6.95$, $P < 0.01$) and repeated exposure (ANOVA, $F_{2,12} = 6.65$, $P < 0.05$) groups (Fig. 6.5). Overall, the type of exposure to ambient salinity did not influence growth (2-ANOVA, $F_{1,24} = 2.30$, $P = 0.143$). However, the growth response was different between the continuous and repeated exposure groups (2-ANOVA, salinity \times exposure, $F_{2,24} = 3.75$, $P < 0.05$). Larvae reared continuously at 28C, 31C and 37C ppt had significantly reduced post-moult size compared to larvae cultured at 34C (Dunnett's, $P < 0.05$). In contrast, in the repeated exposure group, only larvae reared at 28R ppt moulted to a significantly smaller size (Dunnett's, $P < 0.05$) compared to larvae in the control group (34C ppt). The difference in growth response between repeated and continuous exposure groups was particularly marked at 37 ppt (t-test, $t_8 = 9.29$, $P < 0.05$; Fig. 6.5).

6.4.2.4 Routine metabolic rate

A two-way analysis of variance excluding the control group (34A ppt) indicated a significant effect of salinity on the oxygen consumption of stage I larvae ($F_{2,30} = 3.87$, $P < 0.05$; Fig. 6.6). This effect of salinity was statistically marked in both acclimated (ANOVA, $F_{3,20} = 3.58$, $P < 0.05$) and non-acclimated larvae (ANOVA, $F_{3,20} = 4.67$, $P < 0.05$). $\dot{V}O_2$ was significantly different from the 34A ppt control group only in larvae reared at 28A ppt (Dunnett's, $P < 0.05$). Respiratory rates were overall higher in non-acclimated larvae than in acclimated animals (2-ANOVA, $F_{1,30} = 9.79$, $P < 0.01$) but this difference varied significantly over the salinity range tested (2-ANOVA, salinity \times acclimation, $F_{2,30} = 8.78$, $P < 0.01$). This interaction between salinity and acclimation was particularly apparent at 28 ppt where $\dot{V}O_2$ in non-acclimated phyllosoma was significantly higher than in acclimated larvae (t-test, $t_{10} = 42.88$, $P < 0.0001$). Oxygen consumption by non-acclimated larvae and acclimated larvae was not significantly different at 31 ppt (t-test, $t_{10} = 1.19$, $P = 0.301$) and 37 ppt (t-test, $t_{10} = 1.78$, $P = 0.212$).

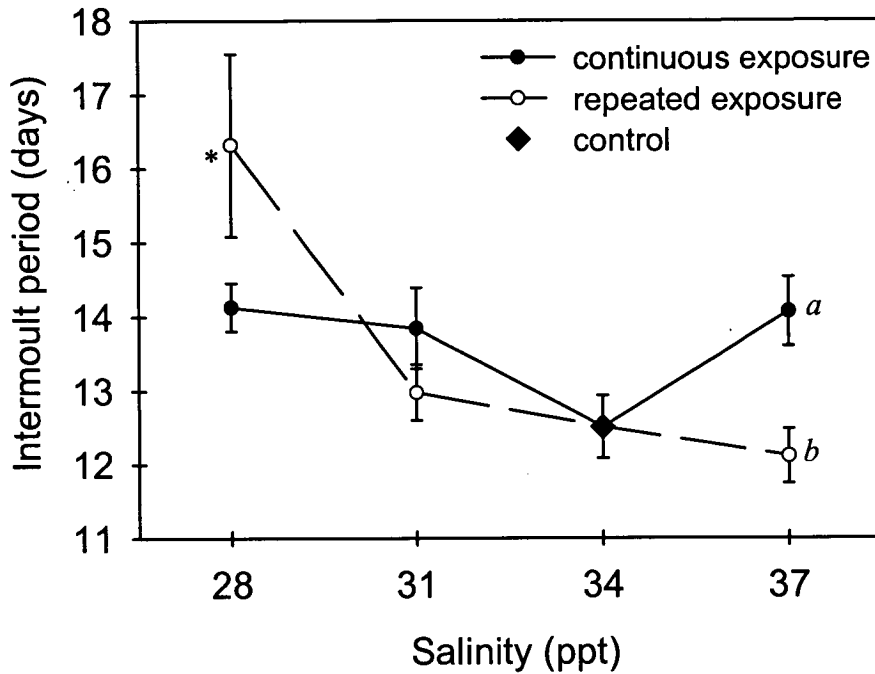


Figure 6.1 Mean (\pm SE) duration of the intermolt period of stage I *Jasus edwardsii* larvae exposed continuously or repeatedly to different treatment salinities. Treatments with different letters differed significantly (t-test, $P < 0.05$). * significantly different from control (Dunnett's, $P < 0.05$).

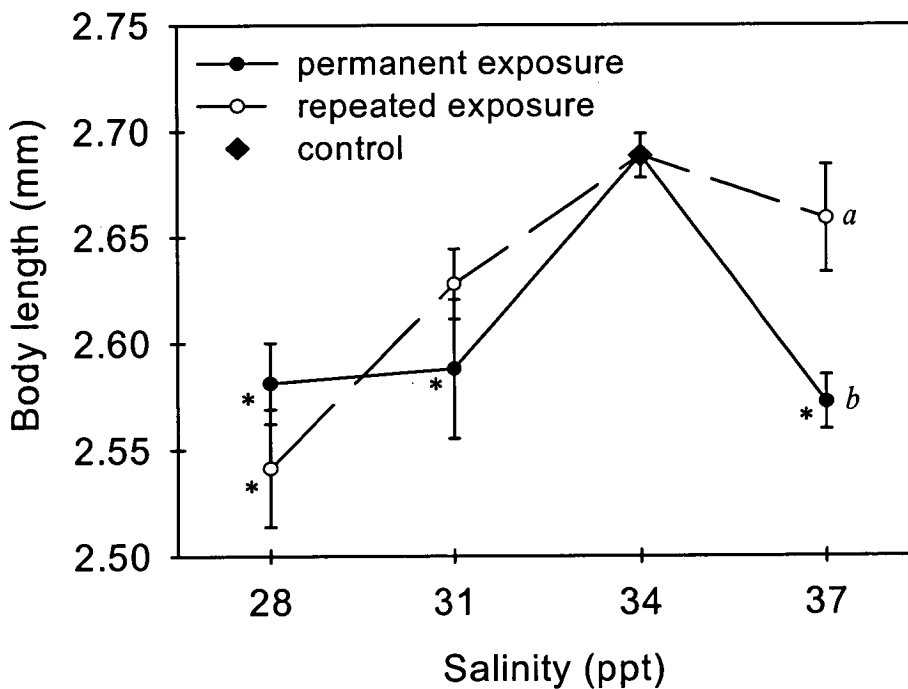


Figure 6.2 Mean (\pm SE) body length at stage II in *Jasus edwardsii* larvae exposed continuously or repeatedly to different treatment salinities during stage I. Treatments with different letters differed significantly (t-test, $P < 0.05$). * significantly different from control (Dunnett's, $P < 0.05$).

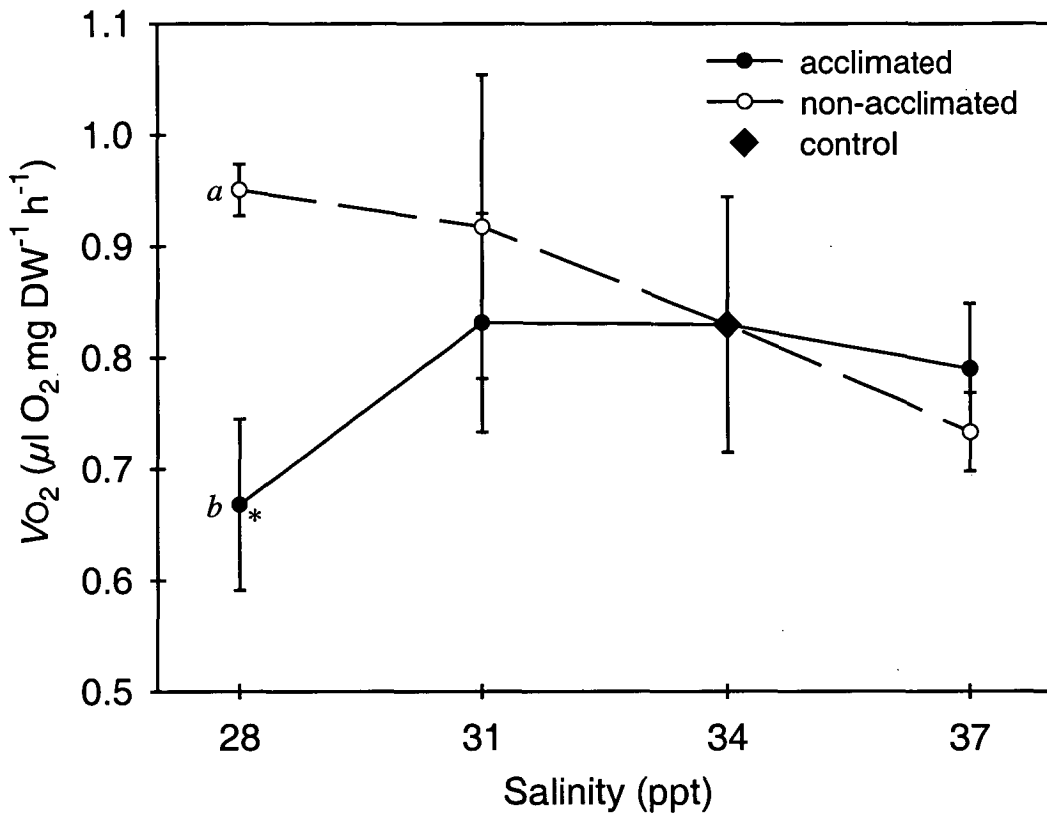


Figure 6.3 Mean (\pm SD) oxygen consumption by stage I *Jasus edwardsii* larvae following a sudden change in salinity from the control condition (34 ppt) and at different acclimation salinities. Treatments with different letters differed significantly (t-test, $P < 0.05$). * significantly different from control (Dunnett's, $P < 0.05$).

6.5 Discussion

6.5.1 *Effects of salinity in newly-hatched larvae*

6.5.1.1 Behaviour

Throughout their oceanic early life history panilurid larvae are able to regulate their depth, seeking optimal conditions for development and/or advective transport (Phillips and McWilliam, 1986; see response to temperature in Chapter 3 and Chapter 4). Newly-hatched *J. edwardsii* larvae are known to be positively phototactic (Chapter 3, Fig. 3.9) but displayed the opposite response (or positive geotaxis) when placed at lower salinities under lights. A gradual increase in negative geotaxis (or positive phototaxis) with increasing salinity suggested a preference for higher salinities and no avoidance of hypersaline waters (37ppt). The avoidance of subnormal salinities was also demonstrated in newly-hatched *Homarus americanus* larvae by Scarratt and Raine (1967) while Latz and Forward (1977) reported on a similar phototaxis reversal in *Rhithropanopeus harrissii* larvae exposed to lower salinities. The behavioural response of *J. edwardsii* larvae to salinity may be of considerable ecological significance for phyllosoma hatching in estuaries or bays where heavy rainfalls could lower the salinity of surface waters. However, the ability of phyllosomas to move along a salinity gradient still needs to be demonstrated.

6.5.1.2 Routine metabolic rate (RMR)

According to Kinne (1971), there are four types of metabolic response to salinity: (1) increased metabolic rate at subnormal salinities and/or decrease at supranormal salinities; (2) increased metabolic rate at both subnormal and supranormal salinities; (3) metabolic rate decreases in salinities below or above normal salinity range; (4) metabolic rate is not affected by changes in salinity. The routine metabolic rates of newly-hatched *J. edwardsii* larvae at different salinities suggest that they follow the type 3 response with a normal salinity at around 31 ppt. However, since behavioural patterns indicated a preference for

salinities higher than 31 ppt, factors other than osmotic stress such as swimming activity, are likely to have influenced the respiratory response over the range of salinities tested. The locomotory compensation for loss in buoyancy in the ~32% of larvae swimming upward in 31 ppt water may have indeed contributed to raising oxygen consumption to a peak under this condition. Therefore, since metabolic rates were higher at low salinities (25 and 28 ppt) than at higher salinity (37 ppt), a type 1 response in routine metabolism may have been masked by the compounded effects of salinity on buoyancy and locomotor activity. As such, the $\dot{V}O_2$ response obtained in the present study is the sum of standard (or basal) metabolism and locomotor activity. Since these two components of RMR respond differently to salinity, data interpretation with respect to the energetic cost of osmotic stress is often ambiguous (von Oertzen, 1984). Given that in newly-hatched *J. edwardsii* larvae, swimming activity did not compensate for the loss in buoyancy at lower salinities, the locomotor activity of larvae placed at 28 ppt can be assumed to be at most equal to the swimming activity of larvae at higher salinities. Consequently, the decline in $\dot{V}O_2$ observed between 28 and 34 ppt and between 25-28 and 37 ppt may be partly attributable to the metabolic cost of osmotic regulation, which is detailed next.

6.5.2 *Effects of salinity in stage I phyllosoma*

6.5.2.1 *The basis of osmoregulation in invertebrates*

Truly marine invertebrates are generally stenohaline and their body fluids are nearly isosmotic with the environment (Willmer *et al.*, 2000). Consequently, in most marine invertebrates, and especially in organisms devoid of external osmoregulation organs (i.e. gills) as in phyllosoma, osmoregulation occurs chiefly at the cellular level. Due to the accumulation of metabolites, cells always need to maintain a degree of osmoregulation to control swelling. This is achieved through passive diffusion of solutes (osmotic effectors) across the membrane, ion-coupled transporters (e.g. symporters and antiporters), and active transport (e.g. the sodium pumps) (Willmer *et al.*, 2000). Osmotic effectors include inorganic ions (e.g. Na^+/K^+) and substances referred to as ninhydrin-positive substances (NPS) (Pierce, 1971) which are essentially comprised of free amino-acids (Livingston *et*

al., 1979; Stickle *et al.*, 1985). NPS are of fundamental ecological significance to marine organisms because they allow cells to regulate their volume while maintaining a stable ionic balance. In invertebrates, active ion transport is thought to be the primary response to an osmotic shock while the accumulation or output of NPS is a longer term osmoregulatory process (Silva and Wright, 1994; Willmer *et al.*, 2000). Regardless of the type of osmotic effectors used in cell volume regulation, an osmotic stress will incur extra energy cost to a marine organism. In hyposaline water, energy will be lost through the active transport of inorganic ions and NPS (Willmer *et al.*, 2000) and through the loss of NPS synthesised from the catabolism of endogenous proteins (Hawkins and Hilbish, 1992). On the other hand, in hypersaline water, active ion transport and also the cellular accumulation of NPS represent significant energy sinks for invertebrates (Hawkins and Hilbish, 1992). These mechanisms of osmoregulation will be referred to in the following interpretation of the results on the effect of fluctuation in ambient salinity obtained in the present study.

6.5.2.2 Constant and fluctuating salinities

The survival of stage I *J. edwardsii* larvae was uniformly high over the range of salinities tested (i.e. 28 to 37 ppt) and in the two exposure groups. In addition, the exposure to steady concentrations ranging from 28 to 37 ppt did not significantly influence the rate of development. However, growth during stage I was affected at sub and supranormal constant salinities (i.e. 28C, 31C and 37C ppt) compared to the control group at 34 ppt. Similarly, slight shifts from optimal salinity (less or equal to 5 ppt) were reported to impair growth in marine crustaceans such as *Pandalus borealis* (Wienberg, 1982) and in *Metapenaeus monoceros* (Kumlu *et al.*, 2001). Interestingly, the repeated exposure of larvae to subnormal concentrations during stage I did not suppress development and growth at 31 and 37 ppt. However, larvae regularly subjected to shifts in salinity of larger amplitude (i.e. 28R ppt group) were delayed in their development and moulted to a smaller size than larvae in the control group. These results suggest that *J. edwardsii* larvae tolerate repeated shifts in salinities of 3 ppt of magnitude while exposure to shifts of a magnitude of 6 ppt would affect their development. This is not surprising considering that the energetic loss associated with active transport

of ions and compensatory solutes would be proportional to the shift in ambient osmolarity. Furthermore, repeated shifts in ambient salinity might constantly alter the hormone concentration in the body fluid as was reported in fish (Woo *et al.*, 1997) and interfere with the ecdysial processes of phyllosoma.

Growth data obtained in the present study indicated that stage I *J. edwardsii* larvae have a greater tolerance for short-term shifts in ambient salinity than for continuous acclimation within the 31-37 ppt range and particularly at 37 ppt. This difference may be function of the time and energy spent osmoregulating. Indeed, following transfer from one medium to another the time course of osmotic equilibrium has been estimated to be from 0.5 to 6 h in the larvae of several crustacean species (see review by Charmantier, 1998). Consequently, osmoregulatory processes may have been less energetically demanding for larvae in the fluctuating salinity group than for animals in the steady salinity group in which regulation was continuous. However, this assumption is not valid for larvae reared at 28 ppt. In fact, at 28 ppt, development was faster and larvae tended to be larger under continuous exposure than in a fluctuating salinity environment (i.e. 28R ppt). This response at lower salinity might indicate a shift in the cellular osmoregulatory process triggered by long term acclimation to hyposmotic conditions. In the bivalve *Mytilus californianus* for instance, Silva and Wright (1994) found that the active transport of inorganic ions was the first process of cell volume regulation occurring during short term response to hyposmotic stress, while losses of NPS were primarily involved in long term acclimation. If the same pattern of response to salinity acclimation occurred in *J. edwardsii* larvae, it would appear to be energetically beneficial only in animals placed under severe osmotic stress (i.e. 28 ppt).

6.5.2.3 Salinity acclimation

Non-acclimated *J. edwardsii* larvae showed a sharp response to salinity with metabolic demand rising with decreasing salinity, and overall, a higher metabolic rate than acclimated larvae. In contrast with the respiratory response of newly-hatched larvae to salinity, the metabolic rate of 4-day-old animals matched Kinne's (1971) type 1 response. This shift in response is likely to be associated with the ontogenic change in behavioural response to light reported in Chapter 3

(Fig. 3.9), since *J. edwardsii* larvae become negatively phototactic two days after hatching in full strength sea water (34–35 ppt). Therefore, at this more advanced stage and without the confounding effect of locomotor activity, changes in oxygen consumption with salinity may be linked directly to osmoregulatory activity. A number of past investigations have attempted to determine some agreement between the metabolic rate of animals and their growth performances under different salinities (Anger *et al.*, 1998; Pechenik *et al.*, 2000). In the present study, respiratory data in non-acclimated larvae provided strong evidence for an elevated metabolic rate under hyposmotic stress. This pattern is similar to the increased oxygen consumption at lower salinities reported in *Neomysis intermedia* (Simmons and Knight, 1975), and to the salinity stress observed in *Cancer magister* megalopas (Brown and Terwilliger, 1999). In stage I *J. edwardsii* larvae, this response may reflect the energetic loss associated with cell volume regulation outlined earlier and would explain the delayed development and reduced growth observed in the 28R ppt group. However, the respirometry results obtained for stage I larvae acclimated at different salinities did not provide convincing physiological evidence for the marked effect of a deviation from ‘normal’ salinity observed on larval growth in the continuous exposure group. This was particularly the case at the 31 and 37 ppt acclimation salinities under which larvae had $\dot{V}O_2$ similar to the $\dot{V}O_2$ of animals in the control group (34 ppt) despite having previously shown a significant reduction of growth under these conditions. Pechenik *et al.* (2000) also reported on a poor relationship between growth rate and energy expenditure in the euryhaline polychaete (*Capitella* sp. I). Working on *Carcinus maenas* larvae, Anger *et al.* (1998) concluded that from measurements of oxygen consumption and food assimilation, only the decline in assimilation could provide a sensible explanation for the decrease in larval growth observed under reduced salinities. Therefore, in *J. edwardsii* larvae, a possible effect of salinity on feed intake, conversion efficiency, or both could have resulted in the reduced growth observed in the 31C and 37C ppt groups. There was nonetheless, a marked decline in $\dot{V}O_2$ observed in *J. edwardsii* larvae acclimated to the 28 ppt salinity. Under this condition, the difference in metabolic rate between acclimated and non-acclimated larvae could in fact explain the delayed development observed under repeated exposure to the same salinity.

6.6 Conclusions

As for most marine invertebrates, the results presented in this chapter indicated that *J. edwardsii* larvae are stenohaline with a weak tolerance for hypo and hyperosmotic stress. Although the survival of stage I larvae was not affected by salinities ranging from 28 to 37 ppt, their long term tolerance may be diminished from the constant exposure to waters shifting only slightly from normal salinity (i.e. ~34-35 ppt). Additionally, repeated shifts from normal salinity within the range of 31-37 ppt did not appear to affect larval development and growth during stage I. The energetic cost of osmoregulation could not be determined accurately through the measurement of oxygen consumption. Therefore, a next step in understanding the long term effect of changes in ambient salinity in phyllosoma should be an integrated physiological approach including the studies of body fluid osmosis, respiration, excretion and feeding. Such detailed investigation would provide the necessary data to assess the effects of constant and fluctuating salinities on a finer scale than the scope of the present study allowed for.

6.7 References

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Chapter 7

Tolerance to ammonia in early larval stages

7.1 Abstract

The total ammonia median lethal concentration (96-h LC_{50}) was determined for stages I, II, III, and IV *Jasus edwardsii* larvae with static water bioassays at 19.0°C. The 96-h LC_{50} for total ammonia (and corresponding NH_3 -N) were 31.58 (0.97) $mg\ l^{-1}$, 45.71 (1.40) $mg\ l^{-1}$, 52.12 (1.59) $mg\ l^{-1}$, and 35.51 (1.01) $mg\ l^{-1}$ at stage I, II, III, and IV, respectively. Stage II larvae were cultured through to stage III at total ammonia concentrations of 0.52 (control), 1.37, 3.83, 6.28, and 9.49 $mg\ l^{-1}$. The intermoult period of stage II larvae significantly increased at and above a total ammonia concentration of 6.28 $mg\ l^{-1}$. Therefore, the no-observable-effect-concentration (NOEC) was 3.83 $mg\ l^{-1}$ (0.12 $mg\ NH_3$ -N l^{-1}) in stage II phyllosoma. The NOECs at stage I, III, and IV were estimated by dividing the 96-h LC_{50} obtained at each stage by the acute:chronic ratio (i.e. 96-h $LC_{50} \div$ NOEC) determined experimentally in stage II larvae. The estimated NOECs for total ammonia (and corresponding NH_3 -N) were 2.65 (0.08) $mg\ l^{-1}$, 4.37 (0.14) $mg\ l^{-1}$, and 2.98 (0.09) $mg\ l^{-1}$ for stage I, III, and IV larvae, respectively.

7.2 Introduction

The water quality in aquaculture systems commonly deteriorates with the accumulation of nitrogenous wastes originating from intensive feeding regimes. Ammonia may be liberated in the aquatic environment from the decay of uneaten food and as the result of catabolism in cultured organisms (Tomasso, 1994). Ammonia is the main nitrogenous excretory product in aquatic animals (Willmer *et al.*, 2000). Total ammonia (Ammonia-N, or $\text{NH}_4^+ + \text{NH}_3$) exists in solution primarily as the ammonium ion (NH_4^+) and the un-ionised NH_3 ($\text{NH}_3\text{-N}$) molecule, the proportions of which are pH, temperature, and salinity dependent. The un-ionised form of ammonia is able to diffuse readily across cell membranes (Fromm and Gillette, 1968) and is considered to be more toxic to aquatic animals than the ionised form (i.e. NH_4^+) (Tomasso, 1994). Ammonia toxicity was reported in all life stages (i.e. larvae, post-larvae, juveniles, and adults) of crustaceans (Armstrong *et al.*, 1978; Chin and Chen, 1987; Kou and Chen, 1991; Young-lai *et al.*, 1991; Lin *et al.*, 1993; Zhao *et al.*, 1998). Shifts in the tolerance to ammonia throughout life stages and also during larval development were common findings amongst these studies. Although chronic toxicity data are essential to the design of aquaculture systems, this aspect of ammonia toxicity in crustaceans has been less extensively studied than acute toxicity (Armstrong *et al.*, 1978; Chen and Tu, 1991; Chen and Lin, 1992; Wasielesky *et al.*, 1994).

The aims of the present study were to define acceptable levels of ammonia for the hatchery propagation of *Jasus edwardsii*. The ontogenic changes in the tolerance of larvae to acute levels of total ammonia were examined from the first to the fourth stage of development. The effect of chronic levels of ammonia on growth was also examined in stage II larvae. Recommendations of acceptable chronic levels of total ammonia and un-ionised ammonia were estimated for stage I, III, and IV larvae from the acute:chronic ratio (Tomasso, 1994) obtained experimentally in stage II phyllosomas.

7.3 Material and methods

7.3.1 Toxicity bioassays

Static bioassays were conducted for 96 h to determine the total ammonia (mg l^{-1}) median lethal concentration (also referred to as 96-h LC_{50}) in mid-stages I, II, III, and IV *J. edwardsii* larvae. Larvae were sampled from mass culture tanks (Ritar, 2001) and placed in plastic jars (220 ml) filled with seawater (mean of daily salinity measurements $\pm\text{SD} = 34.2 \pm 0.8$ ppt) and a buffered stock solution of ammonium chloride (2000 mg l^{-1}) in order to obtain exposure concentrations ranging from 10 to 90 $\text{mg Ammonia-N l}^{-1}$. Jars were stocked with 15, 12, 10 and 10 animals at stage I, II, III, and IV, respectively. Tests were conducted in larvae from 2 broods at each stage and in duplicates for each brood. Jars were randomly arranged in a thermo-regulated water-bath (mean of daily temperature measurements $\pm\text{SD} = 19.0 \pm 0.5^\circ\text{C}$). Larvae were fed daily with on-grown artemia (length: 1.5-3 mm). Dead larvae were removed and counted daily, while live animals were transferred to clean jars and test solution. The actual total ammonia concentration in test solutions was determined daily with the phenol-hypochlorite method (Solórzano, 1969). pH was measured daily and averaged ($\pm\text{SD}$) at 8.04 ± 0.09 . Antibiotic (oxytetracycline hydrochloride, Engemycin 100, Intervet, Australia) was added to seawater (25 ppm) to prevent heavy natural mortality. To account for natural mortality, a control treatment without ammonia was added to each bioassay. Mortality rates observed at each concentration tested were corrected for natural mortality assumed to be independent of treatment and caused by handling and rearing conditions in the control groups with the following Abbott's formula (Finney, 1971):

$$P = \frac{P^* - C}{1 - C}$$

where P , C and P^* are proportions of mortality caused by ammonia toxicity, natural mortality, and total mortality, respectively. The 96-h LC_{50} ($\pm 95\%$ CI) for total ammonia was obtained at each stage using probit analysis

(Norušis, 1990) on the response corrected for natural mortality. The corresponding concentrations of $\text{NH}_3\text{-N}$ were calculated from the tables available in Bower and Bidwell (1978). Differences in the rate of response to increasing dose rates of ammonia from stage I to stage IV were assessed by testing for parallelism (Norušis, 1990) between stages of the linear relationships of the probit transformed response against dose rate. The above analyses were carried out with SPSS 10.0 statistical software.

7.3.2 *Growth trial*

Recently moulted stage II larvae (mean \pm SD length = 2.97 ± 0.06 mm) were sampled from mass culture tanks following rearing through stage I, with the method described by Ritar (2001). Stage II larvae were preferred to first stage phyllosomas in order to avoid the mortality that can occur a few days post-hatching, which is due to poor larval fitness. The larvae were reared through to stage III at nominal total ammonia concentrations of 0, 1, 3, 6, and 10 mg l^{-1} , using the same method described above for bioassays. The actual test concentrations presented in the text were determined from daily measurements of total ammonia in culture seawater with the phenol-hypochlorite method (Solórzano, 1969). There were three replicates per treatment and larvae were initially stocked at a density of 12 per 220 ml plastic jar ($\sim 60 \text{ larvae l}^{-1}$). Mean (\pm SD) temperature, salinity and pH computed from daily measurements were $18.9 (\pm 0.1)^\circ\text{C}$, $34.1 (\pm 1.0)$ ppt and $8.03 (\pm 0.03)$, respectively. Larvae that moulted into stage III were removed from the culture vessels and their image was captured on computer with a Panasonic Super Dynamic WV-CP450 video camera attached to an Olympus S240 stereomicroscope for measurement to the nearest $1 \mu\text{m}$ with Scion software. The effect of total ammonia on survival, intermoult period and post-moult body length was assessed by ANOVA after data were tested for normality (Kolmogorov-Smirnov test) and homoscedasticity (Leven Median test). These analyses were carried out with JMP statistical software. The no-observable-effect concentration (NOEC) and the lowest-observable-effect concentration (LOEC) are expressed for total ammonia and un-ionised ammonia.

7.4 Results

7.4.1 *Acute ammonia toxicity*

7.4.1.1 96-h LC₅₀

There was a tendency for the total ammonia 96-h LC₅₀ to increase from stage I to stage III and subsequently decline at stage IV (Fig. 7.1). However, these observations could not be statistically validated since the 95% confidence intervals indicated uniform LC₅₀s from stage I to stage IV. The 96-h LC₅₀ for total ammonia (and corresponding NH₃-N) were 31.58 (0.97) mg l⁻¹, 45.71 (1.40) mg l⁻¹, 52.12 (1.59) mg l⁻¹, and 35.51 (1.01) mg l⁻¹ at stage I, II, III, and IV, respectively.

7.4.1.2 Rate of mortality response to increasing Ammonia-N

There was a significant difference in the rate of response to Ammonia-N (df = 3, $\chi^2 = 34.47$, $P < 0.0001$) from stage I to stage IV (Fig. 7.2). Indeed, sensitivity to total ammonia declined from stage I to stages II (df = 1, $\chi^2 = 20.13$, $P < 0.0001$) and III (df = 1, $\chi^2 = 11.42$, $P < 0.001$), but increased from stage III to stage IV (df = 1, $\chi^2 = 14.64$, $P < 0.0001$). Rates of mortality response to ammonia were similar at stages I and IV (df = 1, $\chi^2 \approx 0$, $P = 1$), and at stages II and III (df = 1, $\chi^2 \approx 0$, $P = 1$).

7.4.2 *Effect of ammonia toxicity on growth*

The survival of stage II larvae to stage III was not affected within the range of total ammonia concentrations tested (ANOVA, $F_{4,10} = 0.58$, $P = 0.683$; Table 7.1), nor did ammonia have an effect on the body growth of stage II phyllosoma (ANOVA, $F_{4,10} = 1.051$, $P = 0.429$; Table 7.1). However, there was a marked effect of total ammonia concentration on the duration of the intermoult period (ANOVA, $F_{4,10} = 11.60$, $P < 0.001$; Fig. 7.3), and the LOEC at which development was significantly longer (LSD, $P < 0.05$) was 6.28 mg l⁻¹ (or 0.19 mg NH₃-N l⁻¹). The NOEC was 3.83 mg Ammonia-N l⁻¹ (or 0.12 mg NH₃-N l⁻¹).

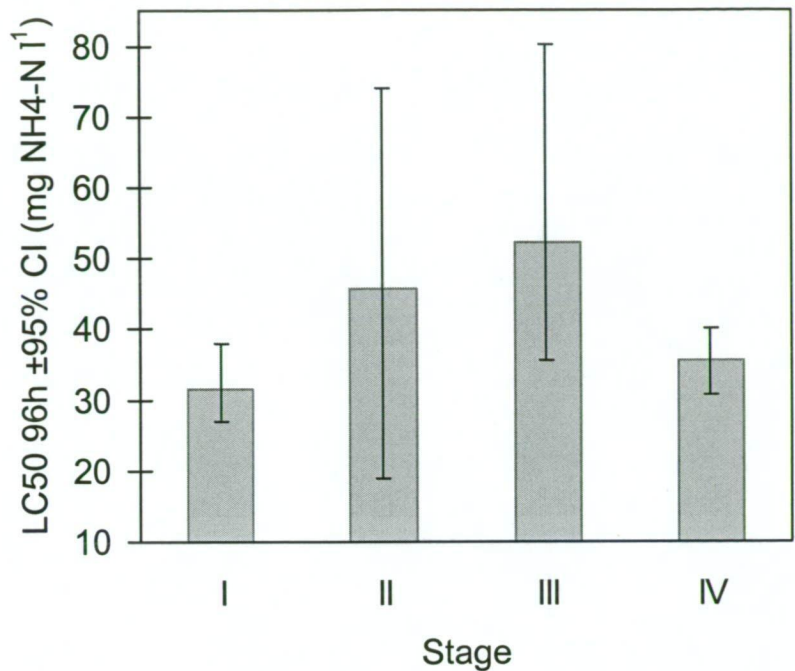


Figure 7.1 96-h LC₅₀ ± 95% CI of total ammonia in stages I, II, III, and IV *Jasus edwardsii* larvae.

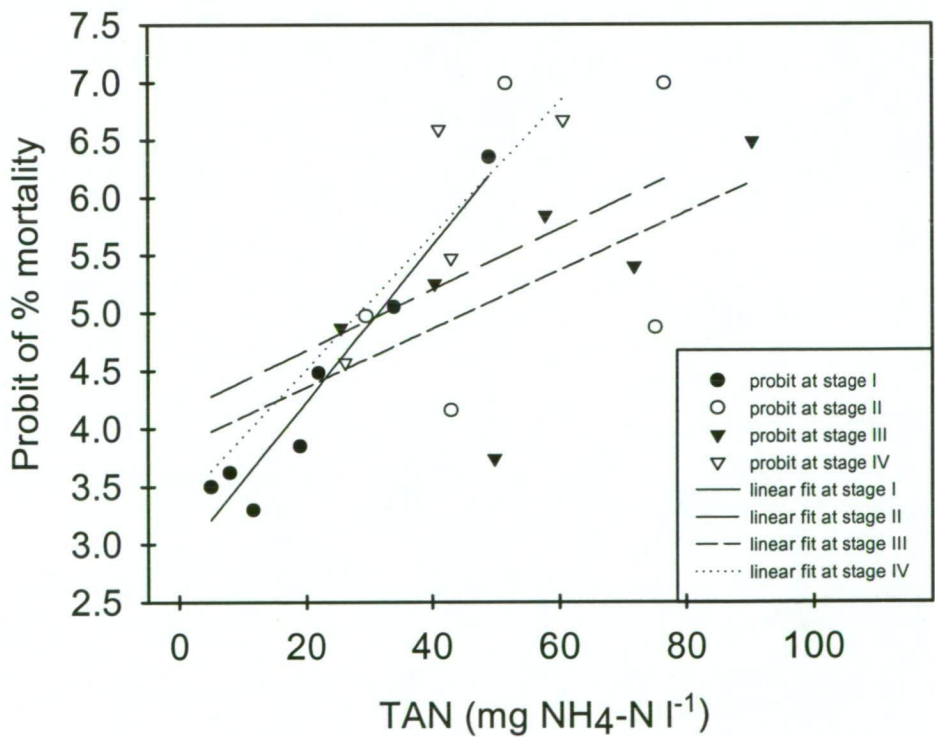


Figure 7.2 Probit transformed mortality of stages I, II, III, and IV *Jasus edwardsii* larvae in response to increasing dose rates of total ammonia. The response was fitted with a linear regression at each stage.

Table 7.2 Post-moult mean \pm SD survival (%) and mean \pm SE length (mm) in stage II *Jasus edwardsii* larvae reared through to stage III at different dose rates of ammonia.

	Total ammonia (mg l ⁻¹)				
Nominal levels	0	1	3	6	10
Measured levels	0.52	1.37	3.83	6.28	9.49
Survival	82.1 \pm 24.2	88.6 \pm 4.6	86.1 \pm 9.6	94.4 \pm 4.8	94.4 \pm 4.8
Length	3.87 \pm 0.00	3.83 \pm 0.03	3.89 \pm 0.02	3.89 \pm 0.02	3.88 \pm 0.01

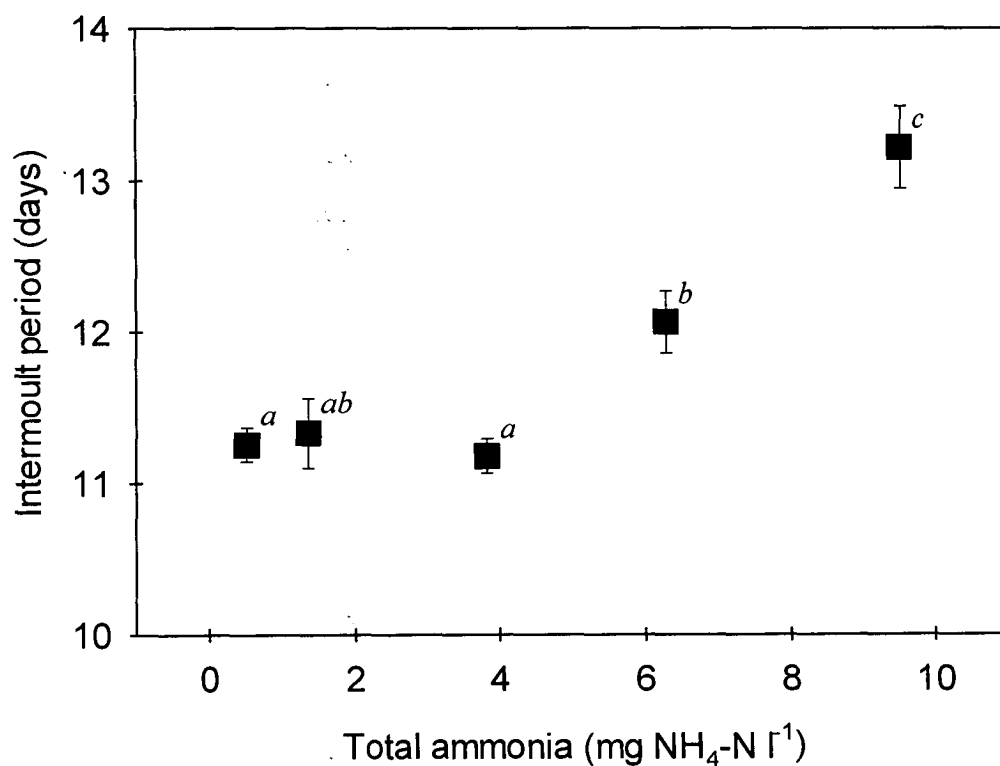


Figure 7.3 Mean \pm SE intermoult period in stage II *Jasus edwardsii* larvae reared through to stage III at different concentrations of total ammonia. Treatments with different letters are significantly different (LSD, $P < 0.05$).

7.5 Discussion

The acute effect of ammonia (i.e. $\text{NH}_4^+ + \text{NH}_3$) was documented on the larval stages of several crustacean species (Chin and Chen, 1987; Young-Lai *et al.*, 1991; Lin *et al.*, 1993; Zhao *et al.*, 1998). The tolerance of *J. edwardsii* phyllosomas to ambient total ammonia and $\text{NH}_3\text{-N}$ was higher than the estimated 96-h median lethal concentration in *Penaeus monodon* nauplii (Chin and Chen, 1987), but similar to the 96-h LC_{50} reported in *Homarus americanus* (Young-Lai *et al.*, 1991) and *Penaeus japonicus* (Lin *et al.*, 1993) larvae. Despite a lack of statistical significance, it is suggested that the tolerance of *J. edwardsii* larvae increased with development from stage I to stage III. Furthermore, from stage I to stages II and III, there was an apparent decline in the rate of mortality response to increasing ammonia concentration. Greater tolerance to raised ambient ammonia with development are common in crustacean larvae and were reported in *P. monodon* (Chin and Chen, 1987), *H. americanus* (Young-Lai *et al.*, 1991), *P. japonicus* (Lin *et al.*, 1993), and *Eriocheir sinensis* (Zhao *et al.*, 1998). However, ontogenic declines in tolerance, such as observed in the present study for stage IV *J. edwardsii* larvae are rare. *J. edwardsii* larvae do not possess gills until 'gill buds' appear in the eleventh and last stage of the larval development (Lesser, 1978). Therefore, an ontogenic change in the organisation of the organs of osmoregulation is not likely to explain the increased sensitivity to ammonia at stage IV. Rearing techniques for *J. edwardsii* larvae are continually refined to improve survival and growth and, suboptimal conditions during mass culture in the present study may have contributed to reduced larval fitness by stage IV (i.e. about 35 days of rearing), thus diminishing their ability to cope with stress.

The effect of ammonia on the growth of crustaceans has rarely been studied in species other than penaeid and palaemonid prawns (Armstrong *et al.*, 1978; Chen and Tu, 1991; Chen and Lin, 1992; Wasielesky *et al.*, 1994). Reduced weight gain and body length increment (Armstrong *et al.*, 1978; Chen and Tu, 1991; Chen and Lin, 1992) are commonly reported as the effects of

ammonia on crustacean growth. In *J. edwardsii* larvae, however, increasing total ammonia concentration had no effect on body growth but delayed development at stage II. Comparatively, Lin *et al.* (1993) observed a slowing of the moulting frequency accompanied by a reduction in growth in *P. japonicus* post-larvae exposed to increasing concentrations of ambient ammonia. Previous workers have conducted growth trials over longer periods of eight weeks (Chen and Tu, 1991) and 30-60 days (Chen and Lin, 1992), or examined animals with high moulting frequency (Armstrong *et al.*, 1978). Therefore, experiments conducted over several larval stages in *J. edwardsii* could result in reduced weight gain or length increment, lowering the NOEC obtained for stage II larvae in this study.

Although the mechanisms of ammonia toxicity in crustaceans remain hypothetical, there has been a number of valuable contributions on the topic (reviewed in Colt and Armstrong, 1981 and Tomasso, 1994). Relevant to the present study, are the reports of reduced excretion in *Callinectes sapidus* (Mangum *et al.*, 1976; as cited by Colt and Armstrong, 1981) and *Macrobrachium rosenbergii* (Armstrong, 1978; as cited by Colt and Armstrong, 1981) exposed to the addition of ammonia to the external medium. Colt and Armstrong (1981) hypothesised that since passive diffusion of NH_3 is the major excretion pathway in most aquatic animals, it would become more difficult for animals to excrete under high ambient ammonia. As a result, animals may reduce or stop feeding to limit the internal accumulation of nitrogenous waste products. Note however, that *Penaeus chinensis* juveniles are particularly well adapted to fluctuating levels of ambient ammonia since they are able to shift their excretion mode from ammonotelic to ureotelic in conditions of elevated external ammonia (Chen and Lin, 1995). While urea is less toxic than ammonia, its synthesis from ammonia would nonetheless incur extra energetic cost to the animal (Willmer *et al.*, 2000).

Ammonia toxicity has also been attributed to the ammonium ion particularly at high concentrations and/or at low pH (Armstrong *et al.*, 1978). The excretion of nitrogen as NH_4^+ is thought to be in the form of an ion exchange of NH_4^+ for Na^+ within active transport sites (Na^+/K^+ -ATPase or sodium pump) of the cell membrane (Pequeux and Gilles, 1981; Willmer *et al.*, 2000). In larvae of the giant river prawn (*M. rosenbergii*), Armstrong *et al.* (1978) found that the

ammonium ion had a greater affinity for the active transport site than the sodium ion. Consequently, ambient NH_4^+ successfully competed with Na^+ to reduce sodium absorption, thereby diminishing body concentration of this important salt that is directly or indirectly involved in amino acid synthesis, transmembrane movement of amino acids or sugars, and possibly gene expression (Willmer *et al.*, 2000).

At the cellular level, high ammonia concentration can cause the diversion of α -ketoglutarate from energy conversion reactions to detoxification processes. This mechanism has so far been investigated in teleosts only and was extensively reviewed by Tomasso (1994) who cited that the diversion of α -ketoglutarate would cause a 68% loss from the normal rate of energy production by the glycolytic-citric acid cycle-electron transport system pathway.

The ammonia toxicity mechanisms stated above could have each played a role in reducing survival and growth in *J. edwardsii* larvae. A clear understanding of ionic and osmotic regulation throughout larval history of *J. edwardsii* is therefore required to define the primary mechanisms of ammonia toxicity for this species and such investigation was beyond the scope of the present study.

The concomitant study of acute and chronic ammonia toxicity allows for the calculation of an acute:chronic ratio (i.e. 96-h $\text{LC}_{50} \div \text{NOEC}$). An inter-species average of this ratio can provide a rough estimate of acceptable levels of ammonia for growth in species for which only acute ammonia toxicity data are available (Tomasso, 1994). This concept is particularly relevant to larval development in crustaceans since an acute:chronic ratio determined in early larval stages could be used to obtain acceptable ammonia concentrations for growth in subsequent stages for which median lethal concentrations are known. An acceptable level of consistency throughout development with respect to the ontogeny of osmoregulation must yet be verified for the principle to be valid, since the tolerance to ambient osmolarity and ionic strength is likely to change with the development of gills, for instance (Charmantier, 1998). In *J. edwardsii*, a same acute:chronic ratio could be applied from stage I to stage X larvae considering that 'gill buds' appear at stage XI (Lesser, 1978). In stage II *J.*

edwardsii larvae, the no-observable-effect concentration (NOEC) for $\text{NH}_3\text{-N}$ was 0.12 mg l^{-1} , which together with the 96-h LC_{50} for the same stage yield an acute:chronic ratio equal to 11.67, a value that is near the $\text{NH}_3\text{-N}$ acute:chronic ratio of 12 reported for *P. monodon* juveniles (Tomasso, 1994). Considering an acute:chronic ratio of 11.93 for total ammonia in stage II *J. edwardsii* larvae, the total ammonia and the $\text{NH}_3\text{-N}$ NOECs can be estimated for stage I, III and IV, and are presented in Table 7.2.

Table 7.2 Estimated or experimental no-observable-effect-concentrations (NOEC, mg l^{-1}) obtained for total ammonia and un-ionised ammonia in stage I, II, III and IV *Jasus edwardsii* larvae. * experimental data.

Stage	NOEC for $\text{NH}_4^+ + \text{NH}_3$	NOEC for $\text{NH}_3\text{-N}$
I	2.65	0.08
II	3.83*	0.12*
III	4.37	0.14
IV	2.98	0.09

These values (Table 7.2) are consistent with the recommendation by Forteath (1990) to maintain crustaceans and molluscs at $\text{NH}_3\text{-N}$ levels no greater than 0.1 mg l^{-1} . Although these values can be considered as safe levels for growing early stage *J. edwardsii* larvae, it is again important to stress that they should be further refined through the assessment of the effect of ammonia on growth in longer term (i.e. more than one stage) exposure trials. In addition to this, further work should focus on the ammonia tolerance of mid and late larval stages in order to define water quality requirements throughout the entire larval development. An understanding of the mechanisms of chronic toxicity of both NH_4^+ and NH_3 would also be valuable to the design of filtration systems for the culture of spiny lobster phyllosomas.

7.6 References

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Chapter 8

General discussion

8.1 Experimental approach: Limitations and scope

8.1.1 *Growth and physiological studies*

From an aquaculture perspective, the effect of varying levels of environmental factors can be assessed through small scale experimental cultures which yield results such as survival and growth rate that are directly relevant to large scale culture situations. Indeed, given survival and growth data, there can be no doubt that 18°C was a better temperature than 14 and 21.5°C to rear *J. edwardsii* larvae through the first three stages of development. In addition, constant darkness was found to be detrimental to grow early stage phyllosoma, while the effect of constant light shifted with development from beneficial at stages I and II, to detrimental at stage III. On the other hand, the scope of physiological assessment spans from the understanding of the effect of environmental factors from a bioenergetics perspective, through to the definition of optimal culture conditions. For instance, the reduced growth observed at high temperature (i.e. 21.5°C) in stage I larvae may be explained by a relative decline in feeding rate combined with increasing energy output through nitrogen excretion at 22°C. In this particular case, recommendations on rearing temperature can be issued from physiological assessment. However, the interpretation of metabolic response to varying levels of abiotic factors can sometimes be ambiguous. For instance, the ontogenic change in response to constant light during growth from stage I to stage III contrasted with the absence of developmental variation in the physiological response to light intensity from hatching to stage V. However, it is important to stress that the culture method and the detailed physiological examination are two fundamentally different approaches. In fact, while a physiological assessment can provide snapshots of the response of animals at any given time during the intermoult period, the response obtained from growing animals is influenced by the condition tested, but also by the nutritional history prior to the developmental stage considered. For instance, the status of the reserves accumulated during previous stages, particularly with respect to the

‘point of reserve saturation’ (Anger and Dawirs, 1981), may influence growth during a given stage by masking or enhancing the effect of environmental factors. This assumption is supported by the findings of Mikami *et al.* (1995), who concluded that palinurid phyllosoma could accumulate reserves when food is available and that when starved, larvae could draw on these reserves to develop through to subsequent stages. Therefore, the snapshot physiological approach, which is not confounded by past nutritional history, is likely to be more suitable than the rearing of larvae through successive stages for detecting ontogenic changes in response.

8.1.2 Scope for a bioenergetic approach

In order to reach an overall understanding of, and to predict the effect of environmental factors on the bioenergetics of phyllosoma, the metabolic response must be detailed at the level of the energy budget model (Anger, 1991):

$$G = F - L - R - U$$

where G is total body growth, F is food uptake, L represents losses by defaecation and leaching of small particles and liquid from food due to inefficient feeding mechanisms, R is respiration (oxygen consumption), and U is nitrogen excretion. The partitioning of energy flows to such level of detail has been described for the larvae of several crustacean species (Mootz and Epifanio, 1974; Levine and Sulkin, 1979; Johns, 1982; Dawirs, 1983; Lemos and Phan, 2001). In the work presented in Chapter 3, only F, R and U were estimated and L was omitted due to the technical difficulties in collecting large amounts of faeces for calorimetric analysis. Consequently, it was not possible to draw and compare energy budgets (i.e. the potential for growth) between *J. edwardsii* larvae reared under different environmental conditions. Additionally, the determination of F is essential in estimating food assimilation ($A = F - L$) which, in zooplankters, may be affected by factors such as temperature (Johns, 1982) and light intensity (Buikema, 1975). However, considering the inherent difficulties in estimating energetic losses associated with feeding mechanisms (Anger, 1991), and due to defaecation (Omori and Ikeda, 1984), food assimilation may have to be deducted as the difference between G and (R + U) estimates. Such approach in balancing

energy budgets is commonly used with larval crustaceans and requires the culturing of experimental animals in order to estimate energy accumulation during the developmental stage studied (Mootz and Epifanio, 1974; Levine and Sulkin, 1979; Johns, 1982; Dawirs, 1983; Lemos and Phan, 2001). Other than understanding the effect of environmental factors on energy flows in *J. edwardsii* larvae, a bioenergetics approach may also be applied in areas such as larval nutrition to compare assimilation between different diets.

8.1.3 Behavioural response to environment

The study of larval behaviour in response to varying levels of environmental factors (i.e. light intensity, temperature and salinity) were valuable in understanding the behaviour of larvae under culture conditions (e.g. ontogenic changes in phototaxis), in defining environmental preferences (e.g. increased negative geotaxis under increasing salinity), and in understanding the behavioural processes underlying physiological responses (i.e. the effect of temperature and light intensity on locomotor activity).

8.1.3.1 Environmental cues and preference

Light, temperature and salinity were all found to influence the depth regulation of newly-hatched or older *J. edwardsii* larvae. However, the type of response obtained for this species and with the methods used (e.g. temperature and salinity), and the range of conditions tested (e.g. light intensity), did not permit the accurate determination of environmental preferences from the study of behaviour alone. The geotactic response of newly-hatched larvae to salinity was, for instance, misleading under hypersaline conditions that were subsequently found to suppress growth. Similarly, the behaviour of newly-hatched and stage I larvae under different temperatures (Chapter 3 and 4) failed to provide supportive evidence for the thermal preference determined through the rearing of phyllosomas. The reasons for this lack of agreement between behavioural studies and results obtained from culturing animals may be due either to the sensory capacities of phyllosomas, or to the techniques used during the present work.

Indeed, given the natural habitat of newly-hatched larvae (i.e. coastal waters), it is not surprising to find that they are only able to sense subnormal salinities rather than supranormal concentrations. Therefore, if newly-hatched larvae are not equipped to sense hypersaline conditions, it is possible that the negative geotaxis of newly-hatched larvae observed at higher salinities (i.e. 34 ppt) will be enhanced by an increase in the density of the external medium (i.e. at 37 ppt). The methods used in the present studies of behaviour may also have limited the scope for behavioural response by exposing larvae to a constant condition each time. For instance, the absence of negative geotaxis at stage II may have been due to a lack of choice (i.e. gradient) within the experimental column. Notwithstanding the fact that thermal gradients at sea would not be so sharp as to be sensed by zooplankters over the 30 cm vertical section of the water columns used here, other environmental stimuli may be essential for the vertical orientation and positioning of *J. edwardsii* phyllosomas. For example, hydrostatic pressure and light intensity can be powerful determinants in the orientation of decapod larvae (Ritz, 1972; Forward *et al.*, 1984; Ennis, 1975; Schembri, 1982; Gardner, 1996; among others). Providing that these parameters can be adjusted, choice experiments would allow for a clear distinction between the environmental stimuli controlling depth regulation in *J. edwardsii* phyllosoma and should be trialed in future work on *in situ* behaviour.

The present study did, nonetheless, provide an insight on the order of importance of light and temperature as factors controlling migration in early-stage phyllosoma. Temperature significantly influenced the vertical positioning of newly-hatched and stage I larvae, but did not appear as the sole factor controlling behaviour in stage I and II phyllosoma placed at temperatures above 12°C. In contrast, the rate of response to light prevailed throughout early development. Consequently, light may be considered as a predominant cue for the control of behaviour in *J. edwardsii* larvae. Additionally, results obtained in the present study tended to show the presence of a light intensity threshold in stage I phyllosomas. In decapod larvae, such a shift in phototaxis generally occurs at very low light intensities (Ritz, 1972; Forward *et al.*, 1984; among others). Therefore, to clearly demonstrate the presence of a reversal in behaviour under declining light intensity in larval *J. edwardsii*, illuminance levels much lower than

the scope of the present study allowed for, will need to be tested. One of the aims of studying the behavioural response of larvae to environmental variables is to define culture conditions that will prevent the occurrence of behaviours leading to cannibalism, increased contact with tank walls, reduced feed intake and other responses that may be detrimental to survival and growth. For that reason, rather than a light intensity threshold *per se*, future investigations should attempt to determine a range of preferred illuminance levels similar to what has been reported for *Panulirus cygnus* larvae (Rimmer and Phillips, 1979). The same approach holds for other abiotic factors such as light spectral composition and angular distribution, temperature, and hydrostatic pressure.

8.1.3.2 The energetics of behavioural response

The locomotor activity of phyllosomas was strongly influenced by factors such as temperature (Chapter 4), light intensity (Chapter 3), and salinity (Chapter 6). Therefore, to understand the metabolic response of *J. edwardsii* larvae to their environment, it is essential to quantify their locomotor activity. While studies of behavioural response in the form of phototaxis and geotaxis can provide some information on the level of activity, they do not lead to the direct estimation of the energetic cost of locomotion. The chemical immobilisation of larvae was assessed in Chapter 4 and yielded promising results. However, this procedure is open to controversy as the exact effects of 2-phenoxyethanol on the different physiological functions of the overall metabolism (Clarke, 1987) are still unknown. Therefore, research may be required to develop further techniques to estimate the energetic cost of swimming in phyllosoma.

Other than allowing for a greater understanding of the metabolic response of *J. edwardsii* larvae to environmental factors, studies of locomotor activity may be necessary for the improvement of larval culture management techniques. In fact, considering that swimming can represent up to 52% (at 15°C) of the energy losses in newly-hatched larvae (Chapter 4), the timing of feeding with respect to diel rhythm of locomotor activity (Sulkin *et al.*, 1979) may be essential in optimising the 'convection requirement index' (CRI = energetic return for metabolic investment). For instance, given that in *J. edwardsii* larvae a light/dark photoperiod resulted in enhanced growth to stage III (Chapter 2) and that feed

intake and swimming activity (together with metabolic rate) are greater in the light than in the dark (Chapter 3), feeding the larvae during the light phase (e.g. at dawn) would optimise the CRI. The improvement of culture system design and larval management techniques for the propagation of *J. edwardsii* will come through further studies on factors such as light, and its parameters (intensity, spectrum and angular distribution). In addition, future valuable contributions may emerge from the study of often overlooked environmental variables such as water currents and turbulence, which have demonstrated effects on the metabolic rate (Alcaraz *et al.*, 1994), swimming activity (Buskey, 1998), and feeding rate (Dower *et al.*, 1997) of zooplankters.

8.2 Tolerance and adaptability

The results obtained throughout this thesis regarding the tolerance of early-stage *J. edwardsii* phyllosoma to the shifts in water quality that can occur in a hatchery situation, represent valuable guidelines for the management of larval rearing facilities. Stage I phyllosomas showed little tolerance for hypoxia with a critical oxygen tension of $4.3 \text{ ml O}_2 \text{ l}^{-1}$ at 18°C (~80% saturation in 34 ppt seawater) which is higher than the minimum recommended levels for aquatic animals (Forteath, 1990; Barnabé, 1991). Larval growth during stage I was suppressed under constant salinities of 31 and 37 ppt and although repeated salinity fluctuation of 3 ppt from normal salinity (i.e. 34 ppt) did not affect development, the effect of long term exposure to repeated osmotic stress has yet to be determined. There is growing evidence to suggest that the ontogeny of crustacean larvae is adapted to the changes in habitat that may occur during larval development. Indeed, ontogenic changes in osmoregulatory processes (e.g. anatomical and/or physiological development) have been reported in several species and these are closely linked to habitat selection (see review by Charmantier, 1998). For instance, the ability of post-embryonic crustacean larvae to hyperosmoregulate at low salinity can be found in the larvae of *Uca subcylindrica* that regularly encounter low salinity conditions in rainfall puddles

(Rabalais and Cameron, 1985). On the other hand, *Penaeus japonicus* larvae progressively increase their tolerance to reduced salinity (e.g. appearance of osmoregulatory epithelia) as they migrate from the open ocean to estuaries and lagoons for settlement (Charmantier *et al.*, 1988; Bouaricha *et al.*, 1994). It is therefore not surprising to find that stage I *J. edwardsii* larvae have a limited tolerance to hypoxia and shifts in salinity, situations which are not likely to occur during their migration from coastal to oceanic waters, and until they migrate back to the coast for settlement.

Maximum safe total ammonia levels were found to be around 2-3 mg $\text{NH}_4^+ + \text{NH}_3 \text{ l}^{-1}$ from stage I to stage IV. The tolerance to ammonia of early-stage *J. edwardsii* larvae is similar to that of larvae in other decapod species such as prawns and clawed lobsters (Young-Lai *et al.*, 1991; Lin *et al.*, 1993). This similarity of tolerance across species may be explained by the toxicity mechanisms of ammonia, thought to be caused largely by the free diffusion of unionised ammonia across membranes (Colt and Armstrong, 1981), which would affect different species in a similar way at a cellular level.

From the results obtained in the present thesis, it is recommended to monitor water quality parameters regularly, or to consider using alarm devices that could be built in larval rearing systems. Future work on the ontogeny of anatomical and physiological development of respiration and osmoregulation, and on the mechanisms of gas and ion exchange in *J. edwardsii* larvae would greatly enhance our understanding of their water chemistry requirements for propagation.

8.3 Perspective for research and aquaculture

Reducing the length of the larval phase has been identified as one of the priority areas of spiny lobster propagation research in Australia (Crear and Hart, 2001). The work presented throughout this thesis indicated that the larval development of *J. edwardsii* could be significantly influenced by all factors tested in growth experiments (i.e. temperature, photoperiod, salinity, ammonia). In addition, light intensity and dissolved oxygen may also affect larval development

through their effect on metabolic rates. Therefore, it may be possible to minimise the duration of the larval phase through the control of environmental factors. However, it is still too early in the research phase to speculate on optimistic figures on the potential rate of development in *J. edwardsii* larvae. Additionally, there has not yet been any cost-benefit analysis carried out for spiny lobster propagation. Considering the comparatively little time required to obtain prawn post-larvae or fish juveniles (Kittaka, 1990; Pillay, 1993), the advances documented throughout this thesis are not likely to lead to the commercial feasibility of a spiny lobster aquaculture industry based on propagation. Other areas of research such as nutrition, the endocrine control of growth, health management, and genetic selection may also enhance the commercial potential of spiny lobster propagation. Although research in each of these areas could significantly contribute to the reduction of the larval phase, this latest is likely to remain protracted compared with species that are currently being cultured commercially (i.e. prawns). Therefore, doubts should be expressed on whether the commercial viability of spiny lobster culture from egg to juvenile will be achieved considering the current labour intensive hatchery techniques used. However, providing that survival can be improved to the level required for supplying seeds to an aquaculture industry, there is still hope for the long-term commercial feasibility of spiny lobster propagation through the development of cost-effective culture techniques. The design of a culture system minimising handling (Illingworth *et al.*, 1997), together with the development of a formulated diet currently under way in Australia and New Zealand (Bradley Crear, personal communication) are along those lines of minimising production costs. Further research in the area of propagation cost-effectiveness may include: (1) the assessment of alternative power supplies to generate heat such as thermal effluent or solar power, (2) the development of minimal labor input husbandry techniques (e.g. to grade larvae), and (3) the endocrine synchronisation of moult.

However, the very first step in larval propagation will be to significantly improve survival. Such a goal will require a long-term research effort in further defining environmental requirements particularly in terms of feeding and assimilation efficiency, in meeting the nutritional needs of phyllosoma that are dictated by their culture environment, and in developing health management practices.

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Appendix I

Early larval stages of the southern rock lobster *Jasus edwardsii*



Figure I.1 Ventral view of a stage I *Jasus edwardsii* phyllosoma.

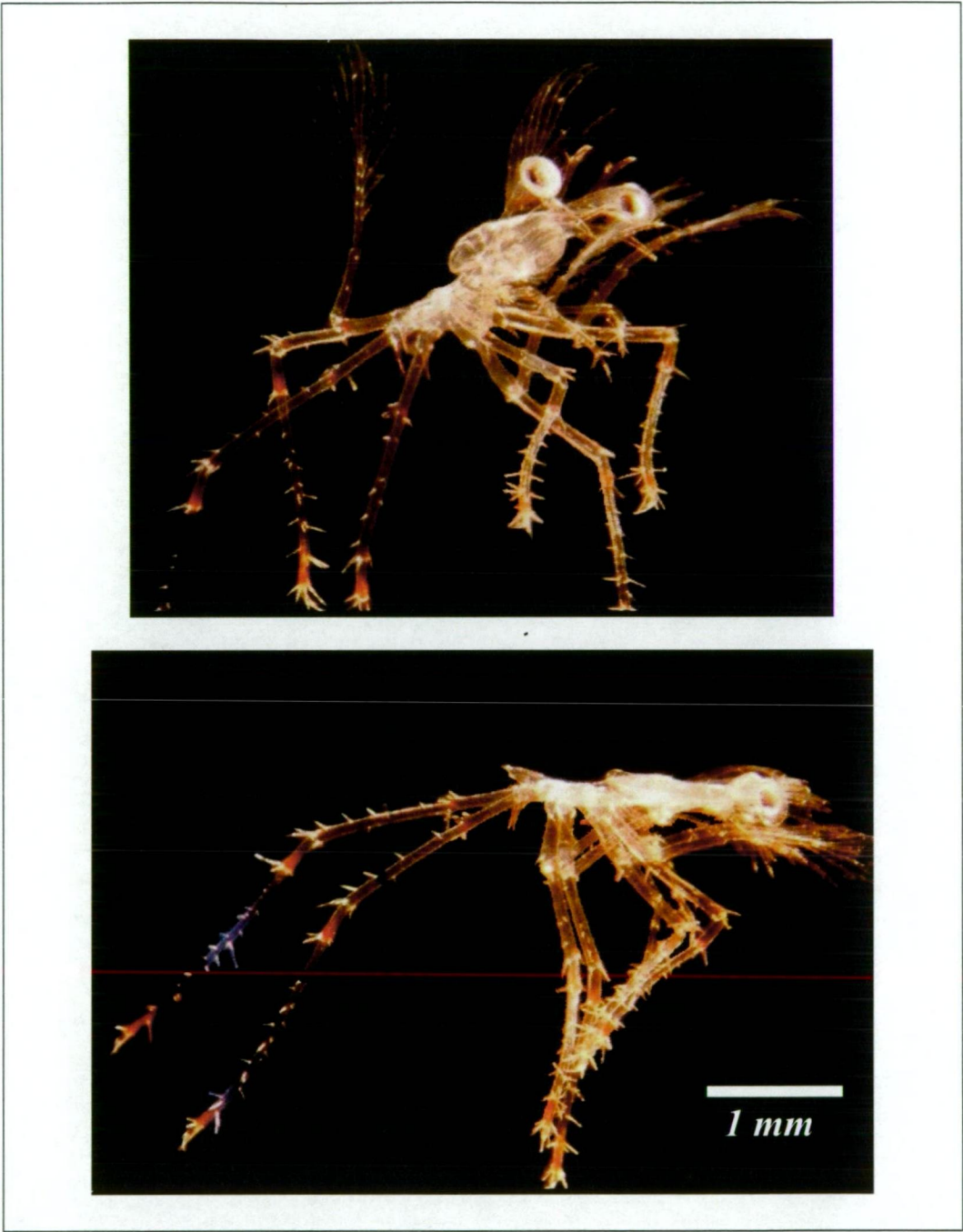


Figure I.2 Side views of a stage I *Jasus edwardsii* phyllosoma.



Figure I.3 Ventral view of a stage II *Jasus edwardsii* phyllosoma.

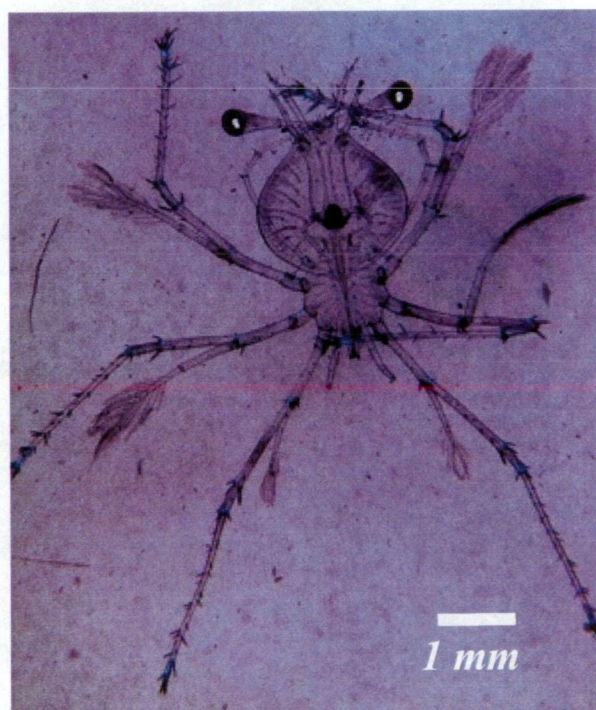


Figure I.4 Ventral view of a stage III *Jasus edwardsii* phyllosoma.

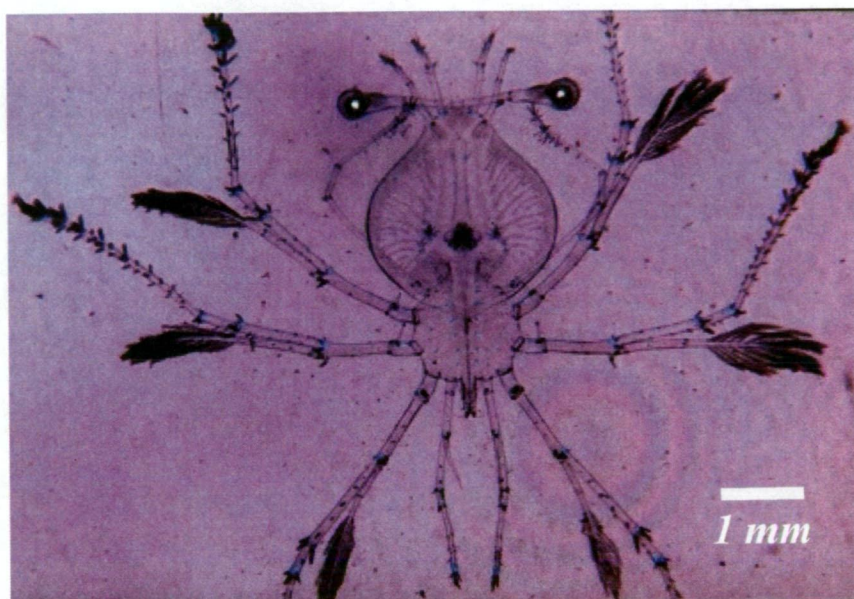


Figure I.5 Ventral view of a stage IV *Jasus edwardsii* phyllosoma.

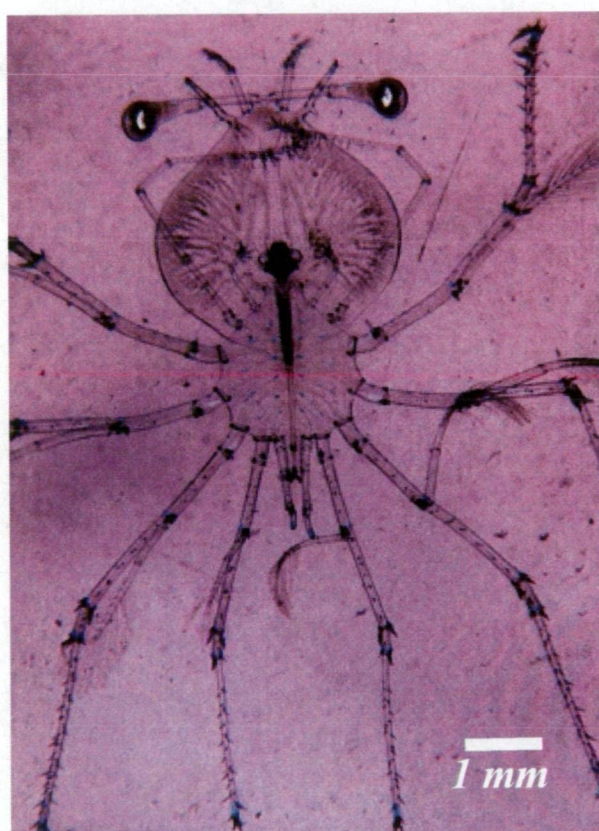


Figure I.6 Ventral view of a stage V *Jasus edwardsii* phyllosoma.

Appendix II

Prophylactic treatment for
experimental static culture

II.1 Introduction and methods

There is an inherent variability in the success of crustacean propagation due to the common occurrence of bacterial diseases (Muroga *et al.*, 1989, cited in Nogami and Maeda, 1992; Igarashi *et al.*, 1991). This variability and/or the loss of replicates may significantly limit the interpretation of data and consequently, the scope of experimental studies. This experiment aimed at defining the oxytetracycline dose rate to use during experimental work in order to minimise mortality due to opportunistic bacteria. The effect of animal density was also tested to determine optimal density in a small static-water culture system.

Newly-hatched larvae collected from one brood in September 1998 were reared through to stage II in 250-ml shallow dark green plastic bowls. The experiment was set up as a Latin square design with treatments of different larval densities (100, 150, and 200 larvae l⁻¹), and oxytetracycline (OTC; Engemycin 100, Intervet, Australia) concentrations (0, 12.5, 25 and 50 ppm). The 12 bowls were randomly placed on a bench under fluorescent lights and at room temperature (~20°C). Larvae were fed daily with *Artemia* nauplii and phyllosomas were transferred every second day to clean bowls and water (+ oxytetracycline at the treatment level). Mortality and moulting were recorded daily. Larvae were collected after moulting into stage II and their body length was measured on a Nikon Profile Projector Model 6C to the nearest 25 µm. The 0 ppm OTC / 100 larvae l⁻¹ treatment was inadvertently lost on the second day of the experiment. Whether data met assumptions of ANOVA or not, the effects of larval density and OTC concentration were determined with ANOVA or the Kruskal-Wallis test (K-W), respectively. Multiple comparison between treatments was carried out with the Least Significance Difference test (LSD). Differences in variance between treatments were tested with the Bartlett's test (Bartlett) of homogeneity of variance (Sokal and Rohlf, 1995).

II.2 Results and conclusions

Although larval density did not significantly affect survival (K-W, $df = 2$, $\chi^2 = 3.68$, $P=0.159$; Fig. II.1.a), it did have a significant influence on the duration of the intermoult period (ANOVA, $F_{2,8} = 12.74$, $P<0.01$; Fig. II.1.c), and on the growth (ANOVA, $F_{2,8} = 5.85$, $P<0.05$; Fig. II.1.e) of stage I larvae. The intermoult period was shorter while growth was greater at densities of 100 and 150 larvae l^{-1} compared with larvae reared at a density of 200 larvae l^{-1} (LSD, $P<0.05$). Oxytetracycline had no effect on survival (K-W, $df = 3$, $\chi^2 = 5.58$, $P = 0.134$; Fig. II.1.b), the duration of the intermoult period (ANOVA; $F_{3,7}=0.04$, $P=0.987$; Fig. II.1.d), and growth (ANOVA, $F_{3,7} = 0.28$, $P = 0.838$; Fig. II.1.f) during stage I. However, the variation in survival was greater in the larvae reared with 0 ppm OTC group than in larvae cultured with 25 and 50 ppm OTC (Bartlett, $P<0.05$; Fig. II.1.b).

On the basis of these results densities of 100 and 150 larvae l^{-1} are recommended to rear *J. edwardsii* larvae through stage I in an experimental static water system. However, lower densities that may further enhance growth should be tested in the future. Oxytetracycline dose rates of 25 and 50 ppm were effective in improving survival and reducing variability between replicates. These results are in agreement with findings by Gardner and Northam (1997). Note that the use of antibiotic is not recommended for the mass culture of phyllosoma since this practice may lead to the emergence of antibiotic resistant bacteria strains. Therefore, research is needed in areas such as pathogens biocontrol (Maeda *et al.*, 1997) in order to improve the health of cultured phyllosomas.

II.3 References

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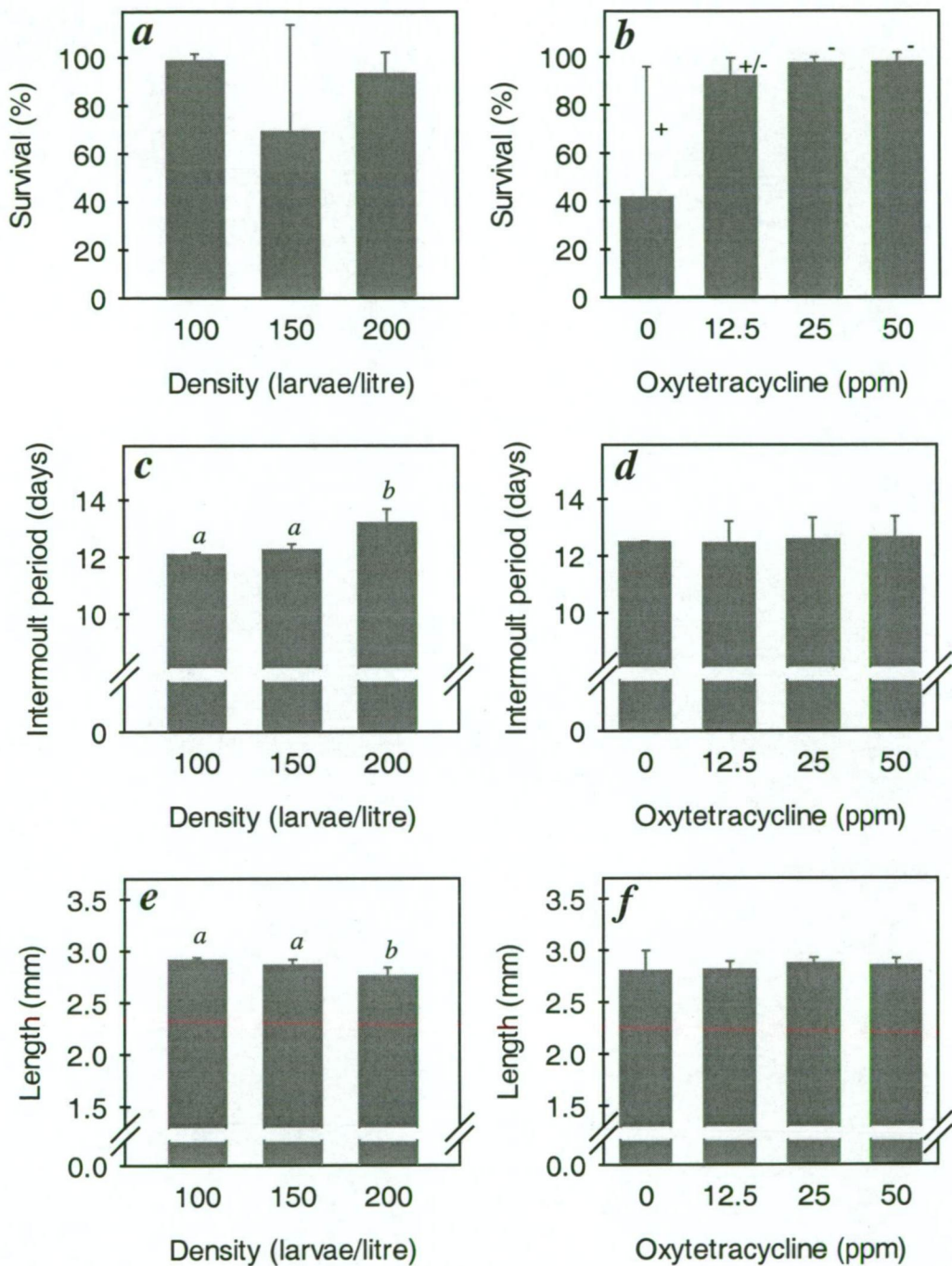



Figure II.1 The effects of larval density and oxytetracycline treatment on the survival (mean \pm SD), intermoult period (mean \pm SE), and length at stage II (mean \pm SE) of *Jasus edwardsii* larvae reared through stage I. Treatments with different letters differed significantly (LSD, $P<0.05$). Treatments with different symbols (+/-) had significantly different variances (Bartlett, $P<0.05$).

Appendix III

Additional data
for Chapter 2
- Microbiology Report -

Note: In the '[CULTURE]' section of the following report, 'FISH #' labelled 'Stage 1 larvae' and 'Stage 2 larvae' were groups of moribund phyllosomas that had been reared at 21.5°C, and 'FISH #' labelled 'From 1 stage 1 larvae' was a single moribund larva sampled in the 18°C treatment. All samples (single larva or groups) were prepared on TCBS medium in petri dishes, which were sent to the Animal Health Laboratory of the Tasmanian Department of Primary Industry, Water and Environment for analysis.

 <p>DEPARTMENT of PRIMARY INDUSTRIES, WATER and ENVIRONMENT</p>	<p>Animal Health Laboratory</p> <p>Department of Primary Industries, Water and Environment ABN 58 259 330 901</p>
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Section Report - Microbiology

Case Id:	98/3290	Serial No:	4975
Submitter:	BERMUDES	Order No:	
		Reference No:	
Received:	10/10/2001	Revision:	0
Finalised:	15/12/1998	Extra Copy	

Owner: TAFI MARINE RESEARCH LABS
GPO BOX 192B
TAS 7000

Species: Rock Lobster **Age:** 0 **Sex:** Unknown

ACCESSION No: 98/3290
4975

SERIAL No:

FISH SPECIES: Rock lobster

[MICROSCOPY]

FISH #	SITE	FINDING	Qty
--------	------	---------	-----

No smears submitted

[CULTURE]

FISH #	SITE	FINDING	Qty
--------	------	---------	-----

Whole animal (labels as received)

Stage 1 larvae	Mixed bacteria including Vibrio alginolyticus	++
----------------	--	----

From 1 stage 1 larvae	Mixed bacteria including V. tubiashii	++
Stage 2 larvae	Mixed bacteria including Vibrio alginolyticus	?

[Qty:] ? occasional + small ++ moderate +++ large

[COMMENTS:] The significance of these findings are hard to assess from this snapshot. V. alginolyticus has been reported as a pathogen in some fish species, however it is also a common component of normal bacterial floras associated with marine animals. V. tubiashii is a recognised pathogen of oyster larvae, but it has not been reported as a pathogen of other marine animals; it has been isolated on several occasions in mixtures of bacteria from a variety of fish and shellfish but the significance of the finding was not clear. The bacteria isolated appear to be consistent with normal(?) colonization. It is possible that the bacteria may be present in excess numbers, but this can only be established through quantitative sampling.

From the note accompanying the samples, a ?permanent? bath of oxytetracycline at 25ppm was used for rearing the larvae. What steps were taken to ensure that the concentration of oxytetracycline was maintained in the water? It should be noted that this antibiotic is moderately labile and it is highly unlikely that a bioactive form of the antibiotic would be present in the water after 13 days if only one addition of antibiotic had been used at day 1.

[DIAGNOSIS:] Normal bacterial flora?

[AUTHORISED:] Dr Jeremy Carson

DATE: 15/12/98

Judith Handler

Ph:

DPIWE Exercises due care in performing all tasks but takes no responsibility for errors associated with sample collection or freight forwarding.

Appendix IV

Is faster bigger?

IV.1 Introduction and methods

Larval size and rate of development are generally used as indices of fitness in studies of larval population. While aquaculture research focuses essentially on comparing mean growth between planned sets of treatments, little emphasis is being placed on larval population structure and dynamics under culture conditions. The aim of this work was to determine the growth pattern of a population of *Jasus edwardsii* larvae reared in a controlled environment in terms of the relationship between intermoult duration and incremental body growth.

Larvae collected from one brood in October 2000 were reared through to stage II in 250-ml shallow dark green plastic bowls. On the morning of hatching about 35 larvae were placed in each of 10 bowls filled with seawater with 25 ppm oxytetracycline (Intervet, Australia). Larvae were fed daily with 1.5-2.5 mm adult *Artemia* following transfer to clean bowls and water (+oxytetracycline). Mortality and moulting were recorded daily. Newly-moulted stage II larvae were collected on the day of moulting (before 1200 h every day) and preserved in formalin prior to measurement of body length to the nearest 1 μm with a computer imaging system (described in Chapter 7). Stage II larvae were preserved in separate containers for each replicated culture vessel and for each day of moulting. Temperature and salinity during the experiment were (mean \pm SD; daily observations) $18.7 \pm 0.1^\circ\text{C}$ and 34.6 ± 0.6 ppt, respectively. Larvae were placed under a 12 h light:12 h dark photoperiod, and the light intensity in the culture vessels during the light phase was $1.1 \cdot 10^{14}$ quanta $\text{s}^{-1} \text{cm}^{-2}$. The Kolmogorov-Smirnov-Lilliefors test (K-S) was used to compare the frequency of moulting at the end of stage I to a normal distribution over the period required for all larvae to moult into stage II. The relationship between length at stage II and the duration of stage I was fitted with a second order polynomial model. The effect of the duration of development during stage I on the size of larvae at stage II was analysed with analysis of variance for unequal sample size and specific differences in larval length between different moult cycle periods were determined with the GT2-method (Sokal and Rohlf, 1995). All statistical procedures were carried out with JMP 3.1 and SPSS 10.0 softwares.

IV.2 Results and discussion

Larval survival (mean \pm SD, $n=10$) through to stage II was $89.2 \pm 7.5\%$. Moulting of phyllosoma from stage I to stage II occurred from day 9 to day 14 post-hatching (Fig. IV.1). The time frequency distribution of moulting events during this period significantly deviated from a normal distribution (KSL, $P<0.0001$), and $80.4 \pm 4.3\%$ mean \pm SE ($n = 10$) of the larvae had moulted by day 11 (skewness of overall data; $g_1 = 1.12$). The length of the larvae declined in a curvilinear fashion as the intermoult period extended (quadratic regression, $r^2 = 0.64$, $F_{2,37} = 32.69$, $P<0.0001$; Fig. IV.2). The size of the larvae was significantly dependent on the duration of development for intermoult periods ranging from 10 to 13 days (ANOVA, $F_{3,32} = 6.88$, $P<0.01$) and larvae moulting after 10 and 11 days of development were larger than larvae moulting 13 days post-hatching (GT2, $P<0.05$).

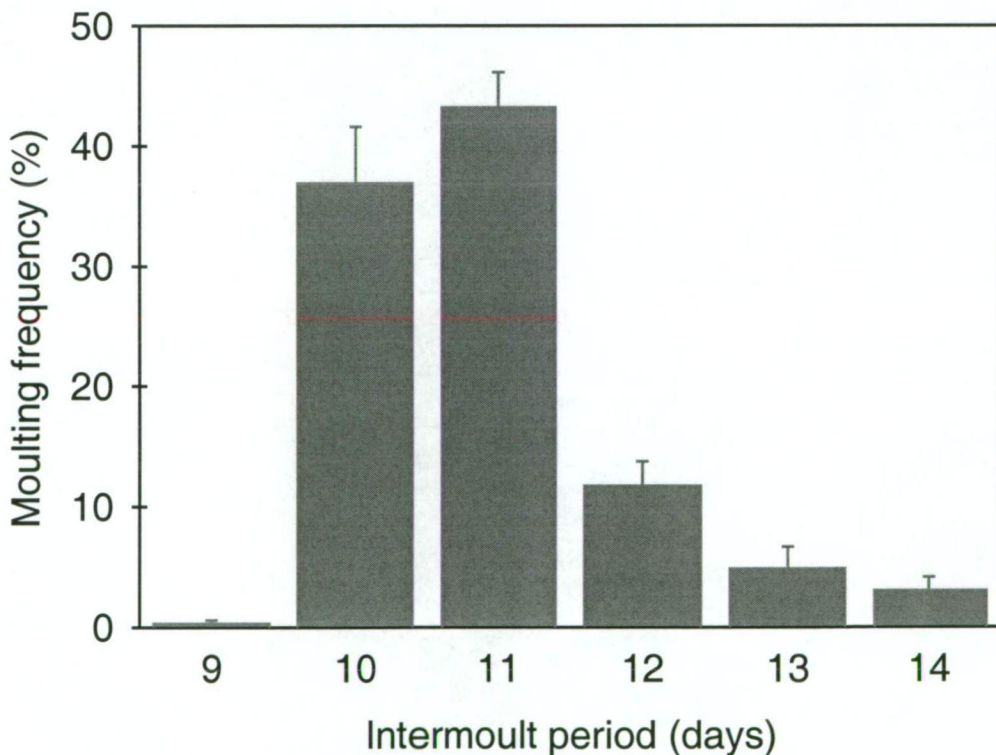


Figure IV.1 Mean (\pm SE) moulting frequency during the 6-day period necessary for all the stage I *Jasus edwardsii* larvae of a population to moult into stage II.

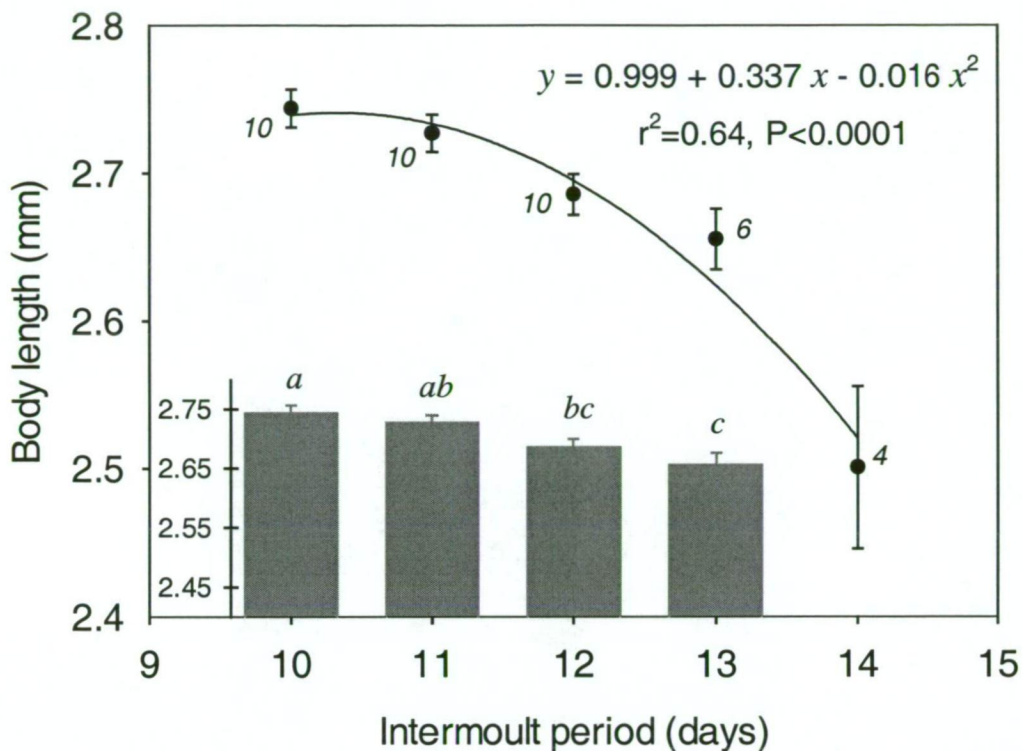


Figure IV.2 Mean (\pm SE) body length in stage II *Jasus edwardsii* larvae reared through stage I in 10 to 14 days. The relationship between intermoult period and the body length of phyllosomas was fitted with a quadratic model. Numbers on each plot indicate the number of replicated rearing vessels in which moulting was recorded on a given day. Bars with different letters differed significantly (GT2, $P < 0.05$).

Hare and Cowen (1997) have extensively reviewed the scientific evidence supporting and refuting the ‘growth-mortality’ hypothesis in planktonic larvae which proposes that as feeding success increases, growth increases and the probability of mortality due to starvation and predation decreases. The same authors identified three mechanisms for the ‘growth-mortality’ hypothesis: (1) if mortality is size dependent, then at a given age, larger individuals will have a higher probability of survival than smaller individuals (ie. ‘bigger is better’ concept); (2) if the probability of mortality is a decreasing function of size, then individuals with higher growth rates will have a lower probability of mortality compared to slower growing larvae (ie. ‘growth-rate’ mechanism); and (3) if juveniles have a lower mortality rate than larvae, then individuals that develop faster and make the larval-to-juvenile transition at a younger age will have higher probability of survival than slower growing animals (ie. ‘stage-duration’ mechanism).

The results of the present experiment indicate that faster developing larvae grow to a larger size than phyllosomas with delayed moulting. However, these findings imply that 'faster is bigger' and not that 'bigger is better' in *J. edwardsii* larvae. Nonetheless, from a numbers perspective and assuming that growth-rate is a fair assessment of fitness, it would appear that size may matter in *J. edwardsii* larvae. This assumption is supported by the fact that the near 80% of larvae that moulted early (from day 9 to day 10) tended to be larger than the phyllosomas with delayed development (moult at day 12-14). In addition, similar observations were made in Chapter 2 and 6 with animals exposed to different temperatures and salinities, respectively. Results from these studies indicated that phyllosomas placed under optimal conditions (ie. 18°C and 34 ppt) grew faster and larger than under suboptimal conditions. Faster development has obvious advantages in benthic invertebrates with a protracted pelagic larval phase in, for example, reducing the risk of predation prior to settlement. Since faster developing larvae are also larger, the growth strategy of *J. edwardsii* phyllosomas may encompass the three mechanisms of the 'growth-mortality' hypothesis cited earlier (ie. 'bigger is better', 'growth-rate', and 'stage-duration' mechanisms). However, these proposed mechanisms need to be verified on the basis of a genetic pool larger than the one brood used in the present study. In addition, it remains essential to assess directly the effect of size on rate of development (ie. instead of the effect of rate of development on size studied here).

The pattern of population growth observed in the present study may have significant implications for the management of mass cultures of *J. edwardsii* larvae. Indeed, the culling of slower (and smaller) growing animals may need to be considered if it is demonstrated that faster developing animals (also larger) have greater probability of survival to metamorphosis than individuals with slower growth rate.

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Appendix V

Light intensity

Unit conversion tables

V.1 Unit conversion methods

In order to allow for comparison of results with past or future studies, the light intensities tested in Chapter 3 on the behaviour (Table V.1) and on the physiology (Table V.2) of early-stage *J. edwardsii* larvae were converted from quanta s⁻¹ cm⁻² to other units commonly used to express light intensity. Note that initial measurements were carried out with a spherical quantum sensor (Biospherical QSL 100) as 'photosynthetic photon flux fluence rate' (PPFFR) also referred to as 'quantum scaler irradiance' or 'photon spherical irradiance'. PPFFR is defined as the integral of photon flux radiance of photosynthetically active radiation (PAR, 400-700 nm waveband) at a point and over all directions about the point (LI-COR, 1991). There is no unique relationship between PPFFR and the 'photosynthetic photon flux density' (PPFD) measurements of incident radiation at a point, which is obtained with a cosine (180°) quantum sensor. Depending on the diffusion of the light source the PPFD may be 1 (eg. collimated beam of normal incidence) to 4 (eg. perfectly diffuse radiation) times the PPFD (LI-COR, 1991).

Conversion between quantum units (quanta s⁻¹ cm⁻² to μmol s⁻¹ m⁻²)

$$1 \mu\text{mol s}^{-1} \text{m}^{-2} = (1 \text{ quanta s}^{-1} \text{cm}^{-2} \times 10,000) \div 6.022 \cdot 10^{17}$$

Conversion from quantum to radiometric units (μmol s⁻¹ m⁻² to W m⁻²)

In order to convert light intensities from quantum to radiometric units (Thimijan and Heins, 1983) a conversion factor was determined for the type of light used in the experiments presented in Chapter 3. The light intensity from a quartz halogen light source placed in a dark chamber was measured in μmol s⁻¹ m⁻² and in W m⁻² using a portable spectroradiometer (Li-Cor, LI-1800). The factor of 5 (473.9 μmol s⁻¹ m⁻² ÷ 94.8 W m⁻²) obtained in this way can be used to convert μmol s⁻¹ m⁻² into W m⁻²:

$$1 \text{ W m}^{-2} = 1 \mu\text{mol s}^{-1} \text{m}^{-2} \div 5$$

Table V.1 Light intensities used to study the behavioural response of *J. edwardsii* larvae (Chapter 3) converted into different units of radiation.

Treatment	Light intensity units						
	quanta s ⁻¹ cm ⁻²	quanta s ⁻¹ m ⁻²	μmol s ⁻¹ m ⁻²	W m ⁻²	W cm ⁻²	mW cm ⁻²	μW cm ⁻²
low	2.9 · 10 ¹⁴	2.9 · 10 ¹⁸	4.843	0.969	9.7 · 10 ⁻⁵	0.0969	96.87
medium	2.5 · 10 ¹⁵	2.5 · 10 ¹⁹	42.068	8.414	8.4 · 10 ⁻⁴	0.8414	841.36
high	1.8 · 10 ¹⁶	1.8 · 10 ²⁰	298.904	59.781	6.0 · 10 ⁻³	5.9781	5978.08

Table V.2 Light intensities used to study the physiological response of *J. edwardsii* larvae (Chapter 3) converted into different units of radiation.

Treatment	Light intensity units						
	quanta s ⁻¹ cm ⁻²	quanta s ⁻¹ m ⁻²	μmol s ⁻¹ m ⁻²	W m ⁻²	W cm ⁻²	mW cm ⁻²	μW cm ⁻²
low	7.7 · 10 ¹²	7.7 · 10 ¹⁶	0.127	0.025	2.5 · 10 ⁻⁶	0.0026	2.55
high	3.9 · 10 ¹⁴	3.9 · 10 ¹⁸	6.476	1.295	1.3 · 10 ⁻⁵	0.1295	129.53

V.2 References

LI-COR, 1991. Radiation Measurements Instruments. LI-COR, Inc., Lincoln, Nebraska, USA.
Thimijan, R.W. and Heins, R.D., 1983. Photometric, radiometric, and quantum light units of measure: A review of procedures and interconversion. *HortScience*, 18: 818-822.

Appendix VI

The effect of starvation on
nitrogen excretion
in stage I larvae

VI.1 Introduction and methods

The aim of this study was to determine the time required for stage I larvae to reach a post-absorptive state in order to determine their 'specific dynamic action' (Jobling, 1983; Chapter 4).

Larvae from two females were reared from hatch in a static culture system of four 220 ml shallow plastic bowls filled with 200 ml of seawater treated with 25 ppm of oxytetracycline (Engemycin 100, Intervet, Australia). Fifty larvae from each brood were stocked in each of the vessels. The water and the bowls were exchanged every second day and larvae were fed to satiation daily with *Artemia nauplii*. Rearing was conducted in the dark, at 16-17°C. In order to monitor the state of starvation of stage I larvae, food was withheld from day 6 post-hatching. At that time (0 h), 10 larvae from each brood were sampled and placed in each of two acid washed vials containing 10 ml of 1 µm filtered sea water + 25 ppm oxytetracycline. The vials were incubated for 24 h at 16-17°C after which time the larvae were removed from the vials and the water samples were preserved at -30°C. The same procedure was repeated at 3, 6, 12, 24, 48, and 72 h after the beginning of the starvation period. Four control vials with no larvae were incubated for 24 h. The frozen samples were analysed for ammonia according to the phenolhypochlorite method described by Solórzano (1969) after less than two weeks of sampling (Parsons *et al.*, 1984). Nitrogen excretion was expressed µg NH₄-N larva h⁻¹ after deduction of ammonia in the control vials. Note that the measurements obtained in this study are not comparable with those presented in Chapter 3 due to the longer incubation period used here. Nitrogen excretion was plotted against starvation period (h) for each of the two broods and fitted with a quadratic polynomial model. The starvation period required for nitrogen excretion to reach a minimum was computed with the following formula:

$$X_{\min} = \frac{-\beta_1}{2\beta_2}$$

where X_{\min} (h) is the time after the start of starvation when nitrogen excretion was the lowest, and β_1 and β_2 are coefficient of the quadratic model (ie. $y = \beta_0 + \beta_1x + \beta_2x^2$).

VI.2 Results and conclusions

The curvilinear nitrogen excretion response of stage I phyllosoma to starvation indicated an initial decline in excretion down to a minimum after 17.5 h followed by a later rise in excretion as starvation continued.

It was concluded that 17.5 h of starvation was required before oxygen consumption could be measured in larvae in a post-absorptive state. The phenomenon of increased nitrogen excretion after a prolonged starvation period was previously reported in zooplankton (Mayzaud, 1973) and in crustaceans (Regnault, 1981). The increased catabolism of protein in starved *J. edwardsii* larvae suggests the ability of the larvae to switch from a lipid to a protein based metabolism in times of starvation (Mayzaud, 1976). However, the greater nitrogen excretion observed in starved larvae may be explained partly by an increased food foraging activity similar to the hunger mediated migration of zooplankton reviewed by Haney (1988). Indeed, as reported in Chapter 3, protein constitutes an important source of energy in actively swimming phyllosomas.

VI.3 References

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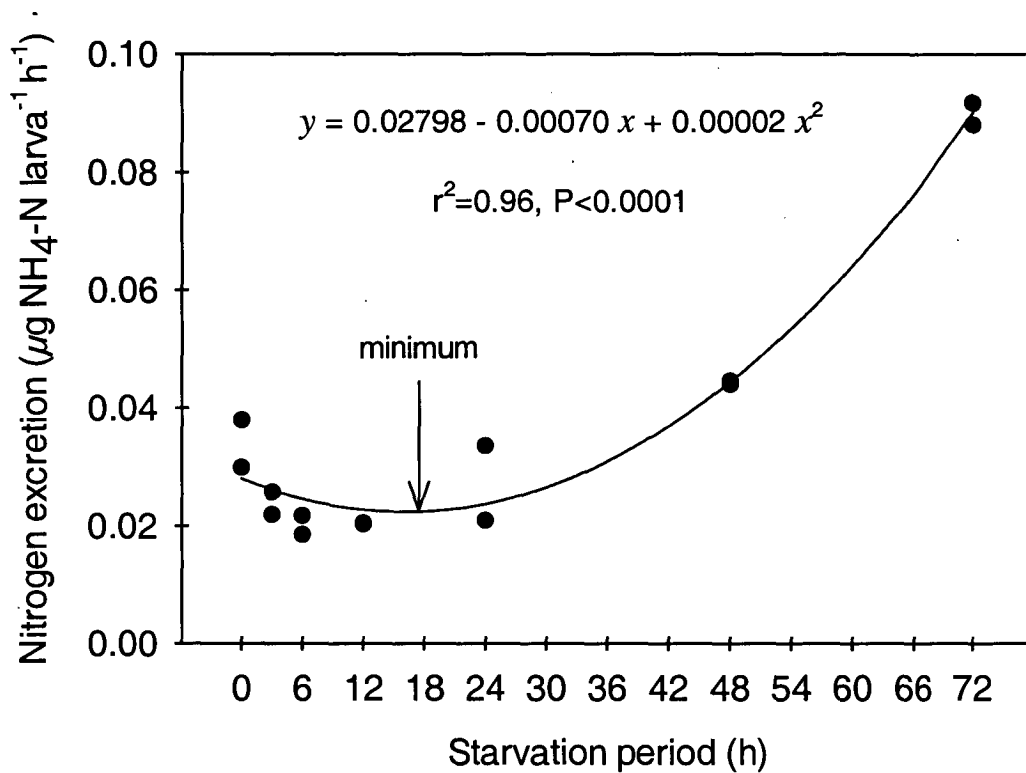


Figure VI.1 Nitrogen excretion of stage I *Jasus edwardsii* larvae subjected to different starvation periods. A quadratic regression was fitted to the raw data obtained from 2 different broods.

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